Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(7): 261
https://doi.org/10.31083/j.fbl2907261

Review

Genetic Identity of Neural Crest Cell Differentiation in Tissue and Organ Development

Stella Aikaterini Kyriakoudi[1,†], Despoina Chatzi[1,†], Iasonas Dermitzakis[1], Sofia Gargani[1], Maria Eleni Manthou[1], Soultana Meditskou[1], Paschalis Theotokis[1,*]

[1]Department of Histology-Embryology, School of Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
*Correspondence: ptheotokis@auth.gr (Paschalis Theotokis)
[†]These authors contributed equally.
Academic Editor: Margarita M Ivanova
Submitted: 22 April 2024 Revised: 16 May 2024 Accepted: 31 May 2024 Published: 22 July 2024

Abstract

The neural crest (NC), also known as the "fourth germ layer", is an embryonic structure with important contributions to multiple tissue and organ systems. Neural crest cells (NCCs) are subjected to epithelial to mesenchymal transition and migrate throughout the embryo until they reach their destinations, where they differentiate into discrete cell types. Specific gene expression enables this precise NCCs delamination and colonization potency in distinct and diverse locations therein. This review aims to summarize the current experimental evidence from multiple species into the NCCs specifier genes that drive this embryo body axes segmentation. Additionally, it attempts to filter further into the genetic background that produces these individual cell subpopulations. Understanding the multifaceted genetic makeup that shapes NC-related embryonic structures will offer valuable insights to researchers studying organogenesis and disease phenotypes arising from dysmorphogenesis.

Keywords: neural crest cells; genes; epithelial-mesenchymal transition; animal models; model organisms; congenital anomalies

1. Introduction

The neural crest (NC) is a specific multipotent population of embryonic cells. It arises from each side of the neural plate and specifically from between the neural and non-neural ectoderm [1]. Neural crest cells (NCCs) undergo migration throughout the embryo by a process known as epithelial-mesenchymal transition (EMT) and differentiate into specific types generating a diverse array of cells [2]. NCCs serve as precursors to a wide range of tissues, that are scattered all throughout the embryonic body. They contribute to many structures in the cranial area, most prominently, to the facial and pharyngeal bones and cartilage, while also taking part in dental development and the morphogenesis of sensory placode derived structures [3–5]. Additionally, NCCs contribute to the shaping of the cardiac outflow tract, while they also give rise to sympathetic and sensory neurons, melanocytes, neuroendocrine cells and contribute to the enteric nervous system.

NC can be divided into several functional domains. In vertebrates, the cephalic or cranial NC originates from the diencephalon to the third somite, playing a role in the formation of bones, cartilage, and connective tissues in the head and neck regions [6]. Additionally, they contribute to the development of pigment cells, localized in the cranial region. Subsequent to cranial NC is the vagal region, a subset of which is the cardiac NC, extending from the otic level to the fourth somite. This region participates in the formation of the heart's outflow tract [3]. The truncal NC, located caudally to the fourth somite, gives rise to peripheral nervous system (PNS) sympathetic ganglia and enteric ganglia, pigment cells (dorsolateral migration), and the endocrine cells found in the adrenal medulla (ventral migration) [7]. Lastly, the sacral NC is responsible for forming the enteric nervous system (ENS), with a contribution from vagal NC.

Unique groups of distinctly expressed transcription factors and proteins orchestrate the differentiation of cells into specialized forms and functions [8–10]. In this review, an attempt is made to deleneate the key genes that participate in the acquisition of NCCs' terminal composition, from rostral-to-caudal region specifiers while presenting miscellaneous derivatives. The data collection aims to present a robust categorization of all the "master genes" implicated in developmental morphogenesis across a plethora of species, with a perspective to address potentional alterations as a causal effect to observed NC-derived malformations.

2. Gene Contribution to NC Regions

NCCs interact with one another while on the move, navigating towards specific locations with their target points being scattered throughout the whole embryonic body. Once they reach their intended destinations, they develop into distinct, specialized cell types, morphing later into the distinct organs that we know of [11]. Being divided in cephalic, cardiac, truncal, and enteric NC, the NC plays a role in developing and contributing to a vast range of systems, such as craniofacial and neck structures, the heart's

Copyright: © 2024 The Author(s). Published by IMR Press.
This is an open access article under the CC BY 4.0 license.

Publisher's Note: IMR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

outflow tract and the enteric nervous system. Here we are going to describe the genes involved in the morphogenesis of these systems.

2.1 Cranial

In the cranial region NCCs play a significant role in forming the majority of the cartilage and bone structures in the skull, contributing in the facial and pharyngeal skeleton [6]. NCCs located at the front of the cranial region contribute significantly to the formation of the frontonasal bones and the membranous bones of the skull. On the other hand, NCCs situated in the posterior part of the cranial region fill in the pharyngeal arches [12]. An interesting scheme that is worth mentioning is the different molecular mechanisms that drive stem cells into entering the cranial rather than the trunk NC family. In particular, ets proto-oncogene 1 (*ets-1*) is of high importance in the distinction between these two cell fates, as it binds to an enhancer region in the forkhead box d3 (*Foxd3*) gene, which is a NC marker, and is required for the differentiation into cranial NC [13]. On the other hand, the same enhancer region needs the *Zic1* gene in order to form trunk cell progenitors. The same regulatory pattern is observed in sex determining region Y (SRY) - box transcription factor 10 (*sox10*) gene expression in the cranial region [14,15]. During the process of delamination in chicken and mice, another crucial difference in proteins that take part is observed, with cranial NC requiring solely sheath interacting protein 1 (Sip1) [7,13,16,17].

Starting from the hindbrain, the migratory pathways of NCCs are organized into distinct streams, with regions lacking NCCs found laterally of the third and fifth rhombomeres, which are the segmented compartments of the hindbrain (r3/5) [18]. The most common model of migration includes three streams starting from three regions of the hindbrain. In Xenopus, the first stream travels around the optic area and enters the first arch, the second stream enters the second arch, while the third stream extends to both the third and fourth arches [19]. Environmental cues guide these streams along specific routes. Specifically, the mesenchyme adjacent to r3 and r5 inhibits NCC movement, obstructing the entry of NCCs into r3 and r5, partially through the actions of erb-b2 receptor tyrosine kinase 4 (Erbb4), which is expressed in r3 and r5 [20,21]. Apart from the repulsive interactions between Eph tyrosine kinase receptors and their ephrin ligands (Eph), the repulsive interactions between the transmembrane neuropilin (Nrp) receptors and their secreted semaphorin (Sema) ligands, also contribute to the shaping of the migratory pathways of NCCs [6,19,22–24]. In zebrafish, the receptors nrp2a and nrp2b are present in migrating NCCs heading towards the first, second and third pharyngeal arches (PA1-3). The ligands sema3f and sema3g are found in areas without NCCs, specifically lateral to r3 and r5 [22]. Also, C-X-C motif chemokine receptor 4a (Cxcr4a) is present in these NCCs, while its receptor, stromal cell derived factor 1b (Sdf1b), lies in the endoderm of the pharyngeal arch. In chicks, *Sema3a* and *Sema3f* are expressed in regions r1, r3, and r5 [23]. In chicks, migrating NCC streams express *EphA3*, *EphA4*, *EphA7*, *EphB1*, *EphB3*, and ephrin B2, while cells surrounding the streams of cranial NCCs express ephrin B1 and EphB2 [25]. In mice, Nrp2 and Nrp1 are present in migrating NCCs targeting PA1-2 and PA2, respectively. Sema3f is present in the caudal midbrain, and both in r3 and r5, while Sema3a is found in r3, where NCCs are absent [24,26]. In Xenopus, Eph tyrosine kinase receptors and their ephrin ligands, also, play here a crucial role. Genes expressed in migrating cranial NCCs include *EphA4* and *EphB1*. These genes are active in the migrating NCCs of the third arch, and in the third and fourth arches, respectively, as well as in the adjacent mesoderm [19]. The ligand ephrin-B2, which interacts with these receptors, is expressed in the nearby NC and mesoderm of the second arch, even before migration begins. The coordinated expression of *EphA4/EphB1* receptors and ephrin-B2 is crucial for limiting the mixing of NCCs from the second and third arches and guiding the third arch NC to its destination. Specifically, *EphA4* is expressed in regions r3 and r5 of the Xenopus embryo's hindbrain, as well as in NCCs adjacent to r5 that are destined for the third arch. NCCs expressing *EphA4* migrate into the third branchial arch. In contrast, *EphB1* expression is observed in a broader domain compared to *EphA4*, encompassing NCCs migrating toward both the third and fourth branchial arches, as well as mesoderm [19]. After NC migration, cells with high levels of *EphB1* expression are confined to the fourth and more caudal arches [27]. Thus, a distinct boundary exists between the NCCs of the second and third arches, as cells expressing *EphA4* and *EphB1* are restricted from migrating into the second arch. Similarly, the presence of ephrin-B2 in the mesoderm of the second arch might contribute to proper migration by restricting the entry of third arch NCCs [19]. In Xenopus *EphA2* is expressed in r4 and also contributes in the distinction of the third and fourth arch NC streams [28]. The most prominent NC genes are depicted in Fig. 1.

Notably significant among cranial NCCs in mouse embryos are chondrocytes. The transcription factor that plays a key role in the differentiation of NCCs into chondrocytes is Sox9 [29,30], which, except for its capacity to regulate its own expression, is strongly affected by WNT signaling in mouse embryos. In accordance with SoxD, it activates a cascade including other genes such as *Agc1* and *Col2a1*. *Agc1* encodes a cartilage proteoglycan that is a marker for the differentiation of NCCs into chondrocytes. The procedure of chondrogenesis relies upon *Agc1* and *Col2a1*, which determine the function and morphology of NC derived chondrocytes. Specifically, in the beginning of chondrogenesis, these genes are upregulated in chondrocytes, however, their production is decreased when terminal differentiation is achieved. Regulation of these two marker

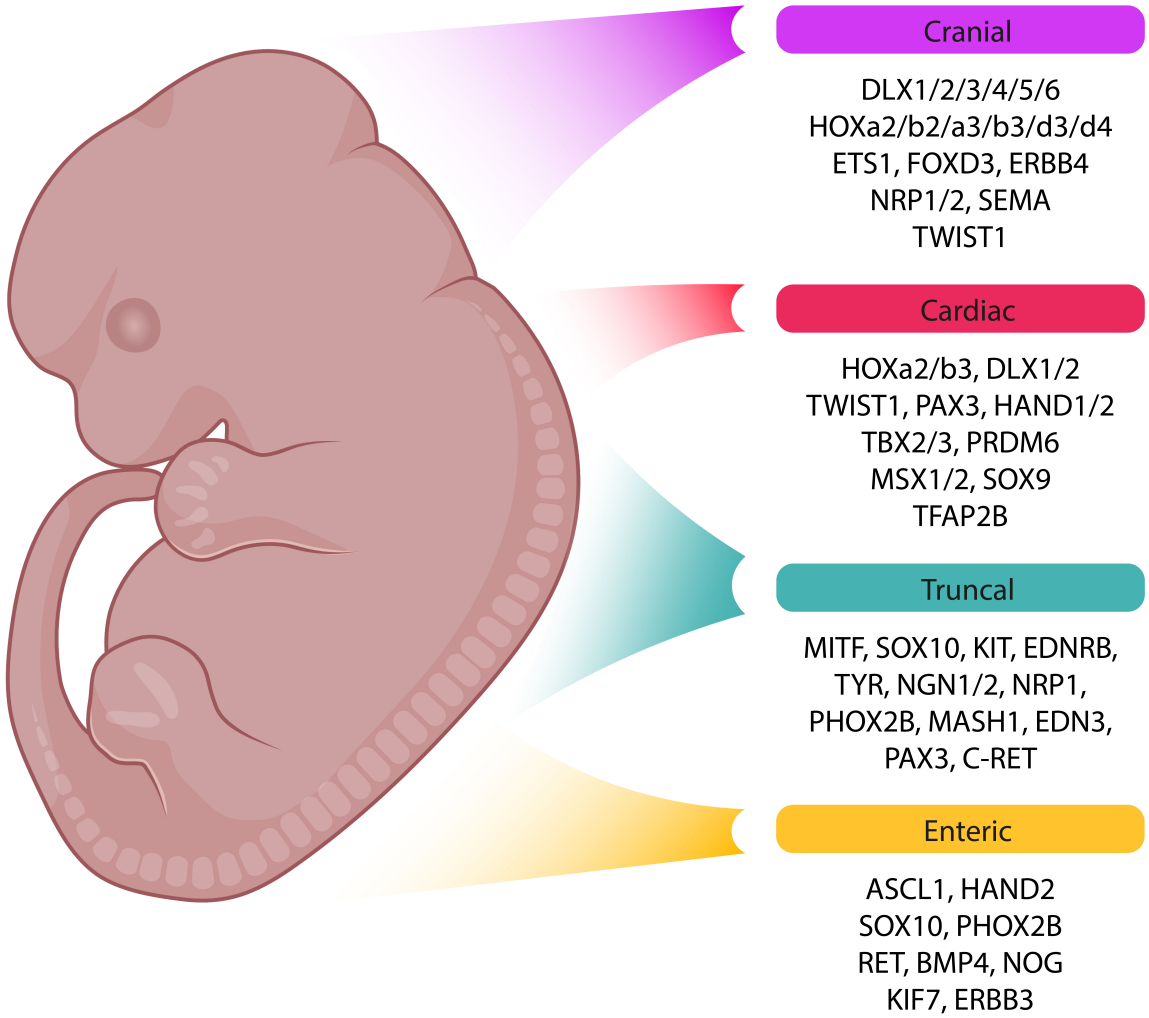

Fig. 1. Key genes expressed in NC subdivisions of the vertebrate embryo. Each color depicts a group of genes expressed in the corresponding region; magenta: cranial NCC, red: cardiac (part of vagal) NCC, cyan: truncal NCC, yellow: enteric (part of vagal and sacral) NCC. Abbreviations: NC, neural crest; NCC, neural crest cell; ASCL, achaete-scute family basic helix-loop-helix (bHLH) transcription factor; BMP, bone morphogenetic protein; DLX, distal-less homeobox; EDN, endothelin; EDNR, endothelin receptor; ERBB4, erb-b2 receptor tyrosine kinase 4; ETS 1, ETS proto-oncogene 1; FOX, forkhead box; HAND, heart and neural crest derivatives; HOX, homeobox; KIF, kinesin family member KIT, KIT proto-oncogene; MITF, melanocyte inducing transcription factor; MSX, MSH homeobox; NC, neural crest; NCC, neural crest cell; NGN1/2, neurogenin-1/2; NRG, neuregulin; NRP, neuropilin; PAX, paired box; PHOX, paired like homeobox; PITX, paired like homeodomain; PRDM, PR/SET domain; RET, Ret proto-oncogene; SEMA, semaphorin; SOX, Sex determining region Y-box transcription factor; TBX, T-box transcription factor; TFAP, transcription factor AP; TWIST, twist family bHLH transcription factor.

genes in chondrocytes requires the presence of both *Sox9* and *Sox5/6*. In mesenchymal cells and NCCs that are going to differentiate into chondrocytes, such marker genes are detected in low levels, as *Sox5/6* are missing. This prevents *Sox9* to bind to chondrocyte enhancers and kickstart their transcription. During the process of differentiation, *Sox5/6* are induced and *Sox9* can then bind to the enhancer site. This interaction drives crest derived progenitors into acquiring the fate of chondrocytes [31]. On the other hand, an antagonist transcription factor of Sox9 that suppresses its activity is Osr1, which is found extensively in soft tissues [32]. Moreover, in mice *Sox9* is present in a number of different precursors of the myoskeletal system including tendon, ligament, bone and cartilage [33,34]. The determination of these destinies relies on nearby signaling molecules and transcription factors, such as such as scleracxis, osterix and Runx2 [35,36]. Initially, osteoblastic precursors separate from the *Sox9* group, followed by the differentiation

of tendocytes and ligamentocytes [36]. On the other hand, skeletal muscle development requires other genes and transcription factors [37–40].

In human development, prechondrocytes originating from NCCs, differentiate into chondrocytes and then secrete matrix molecules that will finally form cartilage. Between prechondrocytes and chondrocytes different marker genes seem to be expressed, revealing the transcriptional change that takes place during the process of embryonic chondrogenesis. Particularly, in the initial stages of development genes like *SOX5/6/9*, forkhead box P4 (*FOXP4*), *NKX 3-2*, *GDF5*, *PCDH8/10*, *PTCH1* and bone morphogenetic protein receptor 1 beta (*BMPR1B*) are transcriptionally active [41–43]. These genes have, also, been detected in mice and chick embryos as well. As prechondrocytes matured into periarticular chondrocytes, there was a gradual reduction in the expression of various mesenchymal genes such as *NCAM1* (encodes CD56), *MCAM* (encodes CD146), *CDH2* (encodes N-cadherin), *CD24*, and, to a smaller degree, *ALCAM*. From these molecules, CD146 is implicated in the process of EMT during early stages of embryonic development, as it contributes to the invasiveness and migration of NCCs. *CD166* is another gene that serves the role of a surface marker mostly in the perichondrium. Accordingly with *CD146*, it enhances both the invasive and migratory character of mesenchymal cells [44–46]. Thus, CD146 and CD166 are gradually decreased as prechondrocytes differentiate into chondrocytes that are immotile terminal differentiated cells. In resting immature chondrocytes at later stages of chondrogenesis, bone morphogenetic protein receptor 1 beta (BMPR1B) is highly expressed in the cells on the surface of articular cartilage, along with leukemia inhibitory factor receptor subunit alpha (LIFR). LIFR is thought to preserve the superficial progenitors of cartilage. These receptors are detected in growth plates after birth, but only for the period that the process of chondrogenesis is active. In mice, BMPR1B is required for chondrogenesis. In terminal differentiated chondrocytes transforming growth factor b1 and 2 (TGF-b1, TGF-b2) and leukemia inhibitory factor (LIF) are highly expressed [47].

In the context of frontal bone development, the ectomesenchyme originating from cranial NCCs is the exclusive source responsible for populating the initial structure of the frontal bone and its underlying dura mater [48]. During this process, TGF plays a vital role as a major factor, promoting the proliferation of NCCs in both the orbital and lateral aspects of the developing frontal bone, even though TGF receptor type II (Tgfbr2) is not required for the survival of osteoblast progenitors [49]. Within the cranial NC-derived ectomesenchyme and cranial sutures, the presence of Tgfbr2 has been identified, demonstrating its significant involvement in human skull development [50]. TGFβ signaling might also be related to FGF signaling, which participates in calvarian development [51]. During frontal bone development, the TGF ligand binds to Tgfbr2, initiating a signaling cascade that regulates proliferation within the cranial NC-derived frontal primordium during the morphogenesis of the skull. These receptors, belonging to a transmembrane serine/threonine kinase family, subsequently influence downstream substrates SMAD2 and SMAD3 [52–54]. Notably, in the context of osteoblast differentiation, TGF signaling specifically impacts the calvarial aspect of the developing frontal bone and not the orbital aspect [49]. Both distal-less homeobox (*DLX*) and *TGF* are crucial in controlling the differentiation of osteoprogenitor cells during skull development, with *Dlx5* serving as a pivotal mediator of osteoblast differentiation in the calvarial region [55,56]. Twist family basic helix-loop-helix transcription factor (*Twist1*) also acts as a regulator of osteoblast differentiation in both mice and humans. During frontal bone development, *Twist1* is expressed in the undifferentiated cranial NC-derived mesenchyme before the initiation of osteoblast differentiation [57]. A key function of *TWIST1* is to suppress the expression of *Fgfr*, thereby inhibiting the osteogenic differentiation of cranial suture mesenchyme. This inhibition maintains the flexibility of cranial sutures during craniofacial development. Afterwards, when osteoblast differentiation is completed, *Twist1* is downregulated. This suppression is mediated by TGF signaling [49].

During the early stages of craniofacial development, the Fibronectin Leucine-Rich Transmembrane (*Flrt*) gene family proteins are crucial for NC migration and craniofacial morphogenesis. In mouse embryos, *Flrt2* is initially highly active in the cranial NCCs, while *Flrt3* exhibits heightened expression in the midbrain with an anterior boundary at the border between midbrain and forebrain [58]. Subsequently, both genes are expressed in the developing pharyngeal region. Specifically, *Flrt2* is expressed in the NC-derived mesenchyme located medially in the developing frontonasal region, closely associated with the expression of *Fgfr2* [59]. Additionally, *Flrt2* expression is present in the vomero-nasal organ, mandibular primordia, and the posterior sections of the secondary palatal shelves. In contrast, *Flrt3* expression was more limited, observed in the mesenchyme beneath the ectoderm of the medial nasal process and beneath the mandibular primordium, with its expression being aligned with the expression of *Bmp4* [60].

The terminal position of cranial NCCs is largely determined in pre-migratory cells by genes belonging in the homeobox (HOX) family. This variety of genes has been excessively studied in Drosophila. A gradient of the expression of these genes is present along the anterior-posterior axis in the pharyngeal arches [61,62]. First of all, the most commonly expressed, homeobox a2 (*Hoxa2*), is active in a very broad region. This region stops at the border of the first and second rhombomeres (r1 and r2). However, NCCs originating from r2 and moving into the first pharyngeal arch (PA1) do not possess any active *Hox* genes, as in PA1 *Hox* genes are not expressed [6]. As a result, the mandibular process has a morphogenetic programme char-

acterized by the absence of these genes' expression. Moving downwards, *Hoxa2* is active in PA2 along with *Hoxb2* in a smaller degree, while in PA3 and PA4, *Hoxa3/b3/d3* expression is present, with *Hoxd4* expression appearing in the last of the pharyngeal arches [6]. When it comes to the dorsal-ventral axis, the latter is organized by distal-less homeobox (*Dlx*) genes, which are six in mammals. In PA1, *Dlx1* and *Dlx2* are active in both the upper (maxillary) and lower (mandibular) jaw structures, covering the area closer to the dorsal part of the embryo. *Dlx5* and *Dlx6* genes, however, are specifically active in the lower jaw and their expression extends near the future joint area between the maxilla and the mandible [55,63]. The domains where *Dlx3* and *Dlx4* genes are expressed are even more restricted, limited to the farthest tip of the lower jaw structure, inhabiting the ventral region of the pharyngeal arches. In the first pharyngeal arch, *Dlx5/6* stimulate and/or sustain the activity of various genes crucial for the formation of the lower jaw structure. These genes include *Dlx3/4*, heart and neural crest derivatives 1/2 (*Hand1/2*), Alx homeobox 3/4 (*Alx3/4*), paired like homeodomain 1 (*Pitx1*), gastrulation brain homeobox 2 (*Gbx2*), and *Bmp7 and Evf2* [6,64]. Crucial genes are listed in Table 1 (Ref. [6,13,19–28,47,55–60,63,65–97]).

2.1.1 Sensory Placodes

An integral aspect of the cranial NCC, is its contribution in the development of the sensory placodes, such as the eye and the ear. These structures are formed from pre-placodal ectoderm, thickenings of the ectoderm in the area of the head, which collaborate with the NC to produce the cranial sensory part of the nervous system [98,99]. Before NC migration begins, both the NCCs and placodes are situated in neighboring areas of the lateral ectoderm. The NCCs are found on both sides of the neural plate, while the placodes partially envelop both the neural plate and the NC [100]. In Xenopus laevis, NCCs go through EMT and display collective cell migration. Meanwhile, placodes positioned in the lateral ectoderm release C-X-C motif chemokine ligand 12 (CXCL12), a widely recognized chemoattractant [101,102]. NCCs, which express Cxcr4, the primary CXCL12 receptor, migrate towards the latero-ventral regions due to chemotaxis guided by its ligand. CXCL12 activates *Rac1*, which plays a crucial role in the extension of cellular protrusions and the formation of focal adhesions in cephalic NCCs, and thus enhances attachment between cells and the extracellular matrix [101,103]. In this whole interaction, matrix metalloproteinase MMP14, which is expressed in Xenopus NCCs [104], might also play a crucial part, through its effect on cleaving the matrix fibronectin [103].

2.1.1.1 Eye. The NC vastly contributes to several structures of the eye, such as the optic cup, lens, ciliary body, iris, cornea, trabecular meshwork, and the primary vitreous [105,106]. A specific group of cranial NCCs migrates and populates the periocular mesenchyme participating in the formation of the eye [107]. TGF signaling aids the NCCs in their migration, guiding them towards the periocular mesenchyme, and controls ocular development. Regulated by TGF signaling, is the expression of *Pitx2* and forkhead box *C1* (*FoxC1*), which produce transcription factors [108,109]. *PITX2* contribution to eye development regards the morphogenesis of structures of the anterior segment of the eye. In chick embryos, Sema3A present in the lens placode restricts the movement of neuropilin 1 expressing NCCs, preventing their migration into the periocular region. This restriction continues until a particular subset of NCCs decreases their *Nrp1* expression [110]. Moreover, paired box 6 (*PAX6*) is one of the necessary genes required to initiate human eye formation and lens differentiation [111]. Its homologous gene *eyeless* in Drosophila can alone initiate eye development [112]. Furthermore, it has been shown that in zebrafish embryos that two separate NC streams enter the optic cup with *pax6* being a necessary guide for their proper migration.

2.1.1.2 Ear. The embryological origin of the ear can be traced back to all three germ layers. The outer ear is formed from the ectoderm, the middle ear is derived from the mesenchyme of the first and second pharyngeal arches, while the inner ear develops from the vesicles of the otic placodes. NCCs originating from the hindbrain and populating the mandibular area of the first pharyngeal arch form Meckel's cartilage, as well as the middle ear bones incus and malleus [113,114]. NCCs are also involved in the morphogenesis of the inner ear by contributing to the cochleovestibular ganglion. During the formation and growth of the inner ear, NCCs migrate from r4 to the otocyst, where they undergo specialization into glial cells of the cochleovestibular ganglion and intermediate melanocytic cells of the cochlear stria vascularis. This process is greatly dependent on *SOX10*, mutations of which cause Waardenburg syndrome, in which deafness is present [115,116]. Moving into PA2, NCCs form the middle ear bone stapes and contribute to a section of the hyoid bone. Precise gene expression domains are essential for the development of these bones. In mice, in PA1, no Hox family gene is expressed. Additionally in the area where the incus is formed, *Dlx1/2* expression is present, whereas in the area where the malleus is formed *Dlx1/2*, *Dlx5/6* are expressed. In the PA2 derivatives, where *Hoxa2* is the only member of its family to be expressed in cranial NCCs [117], the formation of the stapes depends on the expression of *Hoxa2* as well as the expression of *Dlx1/2*. *Hoxa2* is also a defining factor in the external ear formation [6]. A known regulatory factor that interacts with *Hoxa2* is the super enhancer region called *HIRE1/2*, which mediates cell interactions that occur selectively in cranial NCCs from PA2, but not in those from PA1 [117].

Table 1. Most important genes in each neural crest subdivision.

NC subdivisions	Genes	Species they are studied in	References
Cranial	*EPHA4/EPHB1*	Chicken, Xenopus	[19,25,27,28]
	DLX1/2/3/4/5/6	Chicken, Mouse	[55,56,63]
	HOXa2/b2/a3/b3/d3/d4	Chicken, Mouse, Zebrafish, Xenopus	[6]
	ETS1	Chicken	[13]
	FOXD3	Chicken	[13]
	ERBB4	Mouse	[20,21]
	NRP1/2	Mouse, Zebrafish	[22,24,26]
	SEMA	Chicken, Mouse, Zebrafish	[22–24,26]
	TWIST1	Mouse	[57]
	FLRT2/3	Mouse	[58–60]
Cardiac	*HOXa2/b3*	Mouse	[6,65]
	DLX1/2	Mouse	[6,65]
	TWIST1	Mouse	[6,65]
	PAX3	Mouse	[66–69]
	HAND1/2	Mouse	[6,65]
	TBX2/3	Mouse	[65,70]
	PRDM6	Mouse	[71,72]
	MSX1/2	Mouse	[6,65,68,69]
	SOX9	Mouse	[71,72]
	TFAP2B	Mouse	[71,72]
Truncal	*EDNRB*	Mouse	[73]
	EDN3	Mouse	[47]
	PAX3	Mouse	[74,75]
	SOX10	Mouse	[74]
	C-RET	Mouse	[74]
	PHOX2B	Mouse	[76]
	MASH1	Mouse	[77]
	NGN1/2	Mouse, Xenopus	[78–82]
	NRP1	Mouse	[79,83,84]
	MITF	Mouse, Zebrafish	[85,86]
	SOX10	Mouse, Zebrafish	[85–87]
	KIT	Mouse, Zebrafish	[88,89]
	EDNRB	Mouse	[90]
	TYR	Mouse, Zebrafish	[85,91]
Enteric	*ASCL1*	Mouse	[92]
	HAND2	Mouse	[93]
	SOX10	Mouse	[93]
	PHOX2B	Mouse	[93]
	RET	Mouse	[93]
	BMP4	Mouse	[94]
	NOG	Mouse	[95]
	KIF7	Mouse	[96]
	ERBB3	Chicken, Mouse	[78,97]

2.1.2 Teeth

When it comes to the formation of teeth, the latter are formed by the dental lamina, an ectoderm thickening situated in the first PA. The mechanism of the dental formation in mammals is well studied as part of the morphogenesis of the first PA. Cranial NC cells play a crucial role in the formation of mammalian teeth. They serve as the exclusive source of mesenchyme capable of supporting tooth development [5]. These cells not only contribute to most dental tissues but also form the periodontium. The process of tooth development is carefully orchestrated through interactions between cranial NCCs and the oral epithelium [118]. NCC identity regarding dental pattern specification is most likely not determined before the NCCs reach the mandible mesenchyme, but it is determined by their position and the se-

IMR Press

creted signals of the mandible [119]. The first pharyngeal arch is characterized by the absence of *Hox* family gene expression [6]. Although mammalian tooth development is confined to specific regions, namely the first arch and frontonasal process, where *Hox* genes are not expressed, the formation of dental organs in mice appears to occur independently of the Hox patterning program [120,121]. As an example, Hoxa2 overexpression in the area does not disturb the normal formation of the teeth [121]. However, different genes are expressed in each specific area of the mandible. Ectomesenchymal cells in different areas exhibit unique developmental capabilities and will give rise to different types of teeth, thus the different gene patterns [119]. In the proximal molar mesenchyme, Barx homeobox 1 (*Barx1*) and *Dlx1/2* genes are active, whereas in the distal incisal mesenchyme, MSH homeobox 1/2 (*Msx1/2*) and *Alx4* are expressed. In the former, *Barx1*, *Dlx1* and *Dlx2* orchestrate the formation of molars, while in the latter, *Msx1*, *Msx2*, and *Alx4* contribute to the development of incisor morphology [5]. The patterning of the mandible mesenchyme from proximal to distal mirrors the patterning of the overlying oral epithelium. Proximal mesenchymal markers are stimulated by Fgf8, which is secreted by the proximal epithelium [122], while distal mesenchymal markers are induced by Bmp4, secreted by the distal epithelium [123]. Along the rostrocaudal axis, LIM homeobox 7 (*Lhx7*) expression in the rostral mesenchyme, regulated by Fgf8, is complementary to Goosecoid (*Gsc*) expression in the caudal mesenchyme.

2.2 Cardiac

NCCs originating from the occipital region of the embryo belong in a distinct category, called the cardiac NC. This specific subpopulation initiates its migration with the purpose of forming the septum of the cardiac outflow tract and constructing parts of the aortic arches [3]. Along their route, these cells are observed moving from the occipital neural tube, invading the third, forth and sixth branchial arch and later settling into the aortic sac and aortopulmonary outflow tract [124,125]. They exhibit characteristics of both cranial and trunk NCCs, thus the need for their classification in a separate category. Some basic genes expressed in NCCs, while they still reside at the pharyngeal arches, are common with genes expressed in cranial NCCs and include *Hoxa2*, *Hoxb3*, *Dlx1*, *Dlx2* and *Twist1* [6,65]. NCCs destined to differentiate into cardiac NCCs express *Tbx2* and *Tbx3*, which are T-box genes taking part in cardiac development. Later, when these cells arrive at the heart to create its outflow tract, they express not only *Tbx2/3*, but also *Hand1/2*, *Msx1/2*, *Gata3*, forkhead box *f1* (*Foxf1*) and *Isl1/2*. At an even later point, when cardiac NNCs form the outflow tract and the pharyngeal arch arteries by transitioning into smooth muscle cells, smooth muscle cell genes such as *Acta2*, transgelin (*Tagln*), myosin light chain 9 (*Myl9*), myosin heavy chain (*Myh9*), *Rgs5* and *Cnn1* are expressed [65]. Apart from T-box transcription factor genes 2 and 3 (*Tbx2/3*), the gene *Tbx1*, which also produces a T-box transcription factor, shows minimal expression in cardiac NCCs. However, it exhibits robust expression in nearby cells of the pharyngeal arches and is crucial for the proliferation and terminal differentiation of cardiac NCCs [70]. A transmembrane receptor known as Roundabout (Robo) is present on cardiac crest cells and is crucial for the final stage of their migration, as it binds with its Slit ligand, a glycoprotein released into the extracellular space. Slit serves as a navigational signal, helping cardiac NCCs reach their destination [126]. When genes involved in Slit-Robo signaling are mutated, valve abnormalities and septal defects arise [126].

One of the most prominent genes expressed in the cardiac NC is *Pax3*. In mouse embryos, *Pax3* expression is expressed in early NC progenitors and is a marker for cardiac NCCs and essential to their migration to the developing heart [66]. In wildtype mouse embryos, *Pax3* expression is at first undetectable, but it became significantly strong during the point when cardiac NCCs enter the cardiac outflow tract, and then, subsequently, the expression decreased. Cardiac NCCs invade the developing heart around that point of *Pax3* expression. As a result, it can be hypothesized that cardiac NCCs in mice express *Pax3* at least to the point where they enter the cardiac outflow tract [67]. Downstream of *Pax3*, *Msx2* is expressed and its repression is regulated by *Pax3*. However, the related gene *Msx1*, which has been found active in other tissues, has not been found to be expressed in cardiac NCCs [68]. Other cardiac NC markers are the genes *Hoxa-3*, *CrabpI*, *Prx1*, *Prx2*, and *c-met* which were found to be expressed at cells moving through the third, fourth and sixth branchial arches and acquiring their final position in the aortic sac and the cardiac outflow tract [127]. However, all these genes are not expressed at the same level or at the same time points throughout the cardiac NCCs and some of them might also be associated with other types of NC that dwell on localized areas. In more detail, *Hoxa-3* and *Prx1* are expressed at the first and second branchial arches [128], *CrabpI* is expressed only at the first arch at the same time, while *Pax3* is not expressed at all at the first and second branchial arch [69].

Semaphorin signaling is also a recurring factor for cardiac morphogenesis, making its first appearance already from the post delamination stage for cardiac NCCs, as it helps them to find their way from the dorsal aorta to the development area of the embryonic heart and to navigate through the pharyngeal arches [129]. It also is required for cardiac outflow tract septation. In mice, plexinA2 is present in cardiac NCCs as they leave the neural tube. This gene is expressed in the NC, while it still resides in the neural tube, in cells giving rise to the outflow tract of the heart and the aortic arches and also along the migration path connecting the two [130]. Neuropilin 1, which is present in cardiac

NCCs migrating, Neuropilin 2 and other plexin genes have been implicated in being active in the cardiac NC. However, their additional expression in tissues surrounding it, prevents us from reaching a definitive conclusion as to if they can be characterized as marker genes for the cardiac NC [129]. Sema3C and plexinA2, which are present in the roof plate of the neural tube and the compacted mesenchyme of the cardiac outflow endocardial cushions, serve as an appealing directional cue for cardiac crest migration and is found in the cardiac outflow tract [129]. Moreover, necessary signals spanning from the early to the final steps of cardiogenesis are provided and mediated by bone morphogenetic proteins (BMPs) of the TGF family and specifically BMP1, BMP2, BMP4 and BMP7 [131–134]. When migration is completed, cardiac NCCs come together and condense to shape the aorticopulmonary septum. In mice, the TGFβ/BMP signaling group has been associated with this mechanism, including BMP receptor, Alk2, as well as Smad4, that adopts a role of coactivator of this signaling pathway [3,135,136]. *SMAD4* plays a role in both the survival of cardiac NCCs and their differentiation into smooth muscle cells around the fourth pharyngeal arch arteries. SMAD4 is necessary for the development of NC-derived vSMC [135]. It's worth noting that the expression of *SMAD4* in endothelial cells is not necessary for the expression of αSMA or Fn1, but it has been observed that aSMA levels are highly dependent on *SMAD4* expression [135].

A notable marker and morphogenetic gene is PR/SET domain gene 6 (*PRDM6*). The latter functions as a histone methyltransferase in smooth muscle cells, controlling histone modification and gene expression, acting as a transcriptional repressor and enabling other genes [71]. It plays a crucial role in cardiac development, particularly in the migration of cardiac NCCs pre and post EMT. H4K20 monomethylation, a modification mediated by PRDM6, is essential for cell cycle progression and NCC proliferation. In mice, the expression of *PRDM6* is notable in specific cardiac regions like the cardiac outflow tract and the ductus arteriosus, where the smooth muscle cells are derived from cardiac NCCs [71,72]. In cardiac NCCs, *PRDM6* is vital for the closure of the ductus arteriosus and the formation of compacted myocardium. It acts as a regulator for various genes involved in cardiac NCC specification, including *Wnt1*, transcription factor AP 2b gene (*Tfap2b*), and *Sox9*. *Prdm6* serves as a central hub and coordinates the detachment, migration, and differentiation of specific cardiac NCCs, a process dependent on the Wnt1 pathway. *Prdm6* maintains the balance between Bpm4 and Wnt1 signals allowing the NCCs to leave the neural tube and progress through their cell cycle. Downstream of *Wnt1*, *Tfap2b* and *Sox9*, which are NC specifiers, are positively regulated by *Prdm6* and in turn cause the expression of contractile proteins Myh11 and Tagln, which are crucial for differentiation into vSMC [71]. Lastly, some other examples of genes that are typically active in mice premigratory or migrating cardiac NCCs, as well as in tissues located near the pathways of migration are endothelin receptor *A* (*EdnrA*), neurofibromin 1 (*Nf1*), *Cx43*, *Foxc1* and *Foxc2* [137–139]. The last two genes belong to the forkhead family transcription factors and are implicated in mice in the morphogenesis of the arch arteries, the outflow tract and the second heart field [140].

2.3 Truncal

The trunk NCCs originate in the lower part of the embryo and migrate through three distinct routes: a dorsolateral pathway between the ectoderm of the embryo and the somites, a ventrolateral pathway between the somites and a ventromedial pathway between the neural tube and the posterior sclerotome [141]. The trunk NC contributes to the formation of the peripheral nervous system, including the sympathoadrenal system and the enteric ganglia, while also giving rise to melanocytes. The most important genes expressed in the truncal NC derivatives are listed in Table 2 (Ref. [47,73–91,142,143]).

2.3.1 Truncal Neuronal Lineages

The NC gives rise to various neuronal lineages, from the sympathoadrenal system to the enteric ganglia. These neural derivatives of the NC share common expression of some gene markers and transcription factors. As far as the formation of glial and neuronal products is concerned, a majority of genes and signaling cascades seem to be involved. Apart from its significant role in melanocyte formation, *SOX10* is a crucial gene that is responsible for the differentiation of NCCs into neuronal and glial progenitors [144,145]. This can be proved by noting the implications that follow mutations in *SOX10* gene, which can lead to tumors with neural origin, like schwannomas or malignant peripheral nerve sheath tumors [146]. *MEFC2*, another gene that is the direct transcriptional target of SOX10, seems to play a significant role in neuronal development in humans, with its mutations being associated with implications including epilepsy, seizures and intellectual disability [147]. Additionally, the signaling molecule neuregulin determines the fate of peripheral glia while BMP is responsible for differentiation of NCCs into sympathetic neurons [148].

In mice embryos, much discussion has been raised about the distinction between sympathetic and parasympathetic ganglia. The development of noradrenergic characteristics in sympathetic precursor cells begins once they have migrated near the dorsal aorta and is defined by the production and secretion of tyrosine hydroxylase (TH), an enzyme required in catecholamine synthesis [149]. The initiation of sympathetic neuron differentiation is characterized by BMPs production [150], then followed by the induction of multiple transcription factors such as paired like homeobox 2b (Phox2b), Phox2a, Insm1, achaete-scute family bHLH transcription factor (Ascl) 1, Gata2/3, Hand2,

Table 2. Most important genes in the truncal neural crest derivatives.

Truncal derivatives	Genes	Species they are studied in	References
Sympathoadrenal	*NGN1/2*	Mouse, Xenopus	[78–82]
	NRP1	Mouse	[79,83,84]
	PHOX2B	Chicken, Mouse	[76,142,143]
	MASH1	Chicken, Mouse	[142,143]
Enteric ganglia	*EDNRB*	Mouse	[73]
	EDN3	Mouse	[47]
	PAX3	Mouse	[74,75]
	SOX10	Mouse	[74]
	C-RET	Mouse	[74]
	PHOX2B	Mouse	[76]
	MASH1	Mouse	[77]
Melanocytes	*MITF*	Mouse, Zebrafish	[85,86]
	SOX10	Mouse, Zebrafish	[85–87]
	KIT	Mouse, Zebrafish	[88,89]
	EDNRB	Mouse	[90]
	TYR	Mouse, Zebrafish	[85,91]

Sox4 and Sox11 [151]. Research in chick embryos unveiled a step-by-step initiation of gene activity, commencing with *Phox2b* and *Ascl1*, succeeded by *Hand2*, *Phox2a*, and *Gata2* and last but not least genes that are specific for certain cell subtypes [152]. In the process of parasympathetic ganglia formation, which also depends on Ascl1 and Phox2b expression [153], most cells adopt a cholinergic identity, denoted by the presence of choline acetyltransferase and vesicular acetylcholine transporter. Essential for the acquisition of noradrenergic or cholinergic fate is the homeobox transcription factor HMX1. In adrenergic neurons, it conserves the level of TH through the suppression of *Tlx3* and *Ret* and activation of *Trk1*. The opposite cascade that starts with Hmx1 repression is responsible for the differentiation of cholinergic neurons [154].

2.3.1.1 Sympathoadrenal. The autonomic nervous system consists of chromaffin cells found in the adrenal medulla, the sympathetic chain, the organ of Zuckerkandl, and the rest of the prevertebral ganglia. This system is made up of both cranial and trunk NCCs. The former originate above somite five and give rise to structures like sensory, sympathetic, and possibly parasympathetic ganglia, while the latter are formed under somite four and contribute to the development of sympathetic and sensory neurons as well as adrenal chromaffin cells. In particular, common precursor cells give rise to both sympathetic neurons and chromaffin cells of the medulla, while the differentiation between these two cell types happens after delamination [155]. Along with the formation of the sympathoadrenal system, also occurs the formation of melanocytes, with the only difference between them being that melanocyte precursors follow a dorsolateral path when leaving their home site. On the contrary, the other group of NCCs that generates chromaffin cells in the adrenal medulla and cells in the sympathetic and sensory nervous systems migrates ventrally through the intersomitic space or within the anterior sclerotome [156]. The formation of sympathetic ganglia, sensory ganglia, and melanocytes mainly occurs in a sequential manner, meaning that delamination and migration time point plays a substantial part in determining the fate of these common precursors [157]. In greater detail, early migrating NCCs are primarily destined for neuronal and glial roles, whereas NCCs that migrate later tend to become melanocytes. The sympathoadrenal (SA) NCCs are a distinct population of NCCs, deriving between the 18th and 24th somite in most species gathering around the large vessels of the abdomen, where they take their final ganglial form, with a subset of them venturing further down to construct the adrenal medulla and acquiring chromaffin cell characteristics [7,151]. During their descent, in chickens, the Eph/ephrin family plays a role in promoting ventral migration [158], while in mice, Sema3A/Nrp1 signaling serves a similar function, meaning that these two pairs of ligands and receptors direct the path that SA NCCs will follow. NCCs that will form the sympathetic ganglia express *Nrp1* but not *Nrp2*, that seems to be distinctive of cells belonging to the sensory nervous system [79,83,84]. Cells that will become sensory neurons of the dorsal root ganglia (DRGs) express in premigratory level and during their ventral migration neurogenin-1/2 (Ngn1/2), which direct them towards this phenotype [79–82]. Additionally, ErbB/Neuregulin signaling pathway is important, apart from its role in migration of trunk NCCs, for their differentiation into DRG as well as sympathetic ganglia [78].

The anatomical formation most involved in the procedure of SA cells' differentiation, is the dorsal aorta. This vesicle acts as factory responsible for the production of BMPs, including BMP2, BMP4 and BMP7 which are usu-

ally secreted by endothelial cells of the dorsal aorta in chicks [142]. Due to these molecules, the expression of *Phox2a/b*, *Mash/Cash1*, *Insm*, *Hand2* and *GATA2/3* is increased. The BMPs are also essential for the activation of *SDF1* and neuregulin 1 (*NRG1*) in the mesenchyme surrounding the aorta, which also are attractive ques, subsequently influencing the direct control of SA cell morphological development. Afterwards, SDF1 and NRG1 function as chemical signals that attract SA precursors, which directionally migrate towards those secretion sources [148,155,159]. In chick embryos specifically, in some of the ventrally migrating NCCs heading towards the dorsal aorta the receptor CXCR4 is present. This guides them towards SDF1 rich areas [160,161]. AP2 transcription factors also characterize the ventrally migrating NCCs. However, a specific link between their expression and the survival of sympathoadrenal NCCs has not been described [79]. The receptor that is necessary for the binding of BMP molecules is Alk3, which is present in premigratory NCCs. The presence of the transcription factor Phox2b is crucial for the sustenance of sympathetic precursors. Its expression is triggered by BMP signaling and is regulated by *Alk3*. WNT signaling is another regulatory mechanism that takes part in the distinction between sensory and sympathetic neurons. Specifically, canonical WNT signaling, through direct activation of β-catenin, drives NCCs into adopting a sensory fate, a fact being confirmed by the presence of certain gene markers including *ngn1*, *ngn2* and *neuronD*. On the contrary, in β-catenin rich environment, sympathetic neurons are underdeveloped as proven by the disappearance of gene markers like *ehand* and *mash1*. Consequently, canonical WNT signaling determines sensory fate of neural progenitors, while non canonical WNT signaling is necessary for non sensory fates [143]. Even though SA cells have been studied, the differences between the ones that will contribute to the sympathetic system and the ones that will become the adrenal medulla are not well understood. While it has been observed that sympathetic neurons have elevated concentrations of TH and neurofilament (NF) and chromaffin cells only have elevated levels of TH and not NF, these differences are not depicted in their precursors, as these proteins are not expressed in them. What is known is that migrating NCCs with adrenal fates express *Sox10*, *Sox8*, *Scg10* and synaptosome associated protein 25 gene (*Snap25*), even though the last 2 are more common in neuronal cells [79,162].

2.3.1.2 Enteric Ganglia. The enteric ganglia is subset of the ENS, that is derived from truncal, sacral and vagal NC. A trio of genes, *Pax3*, *Sox10*, and *c-RET*, plays a vital role in the development of the NC in the intestine and the enteric ganglia in particular. Precursor cells that express *Pax3* are fated to form enteric ganglia, a morphogenesis event that *Pax3* is crucial for [74]. It is typically present in NCCs before they migrate, but its expression decreases as the cells migrate [66]. This gene can activate the expression of *c-RET*, the second gene in this triad. Pax3 collaborates with the transcription factor Sox10 to initiate the transcription of *c-RET*. In the context of enteric ganglia development in the intestine, the c-RET tyrosine kinase receptor is indispensable, as it is required for their viability and multiplication [75]. *Pax3* and *Sox10* together significantly increase endogenous c-RET expression. For their synergistic activation a specific 45-bp element within the *c-RET* enhancer is required. This enhancer contains adjacent binding sites for *Pax3* and *Sox10* [74]. All enteric ganglia precursors also express the c-RET receptor, necessary for their survival and proliferation, which binds with its ligand, glial cell-derived neurotrophic factor (GDNF) [163,164]. The latter is produced by surrounding mesenchymal cells of the intestine. Apart from Sox10, several other transcription factors, such as Phox2b [76], and Mash1 [77], regulate various aspects of enteric NC differentiation and proliferation. On the other hand, connexin 43 (*Cx43*) promoter directs expression in specific regions, including the dorsal neural tube, dorsal root ganglia, cardiac NC, and other NC-derived tissues [165]. The endothelin B receptor (EDNRB) and endothelin 3 (EDN3) are also essential for distal hindgut ganglia formation [166]. *Ednrb* is typically expressed in enteric ganglia and surrounding mesenchyme [73].

2.3.2 Melanocytes

This cell population is formed mostly by differentiation of truncal NCCs, through a process mediated by a vast number of different genes and signaling cascades. Melanocytes derived from the trunk NC will migrate towards most parts of the embryonic body. In mouse embryos, a transcription factor that is specific and indicative of melanocyte progenitors is melanocyte inducing transcription factor gene (*Mitf*) [85]. This marker gene is responsible for the survival of melanocyte progenitors, by regulating antiapoptotic factors like *Bcl12*. It also contributes to cell multiplication, by controlling *INK4A/p16*, *p21*, *Tbx2*, and cyclin-dependent kinase (*CDK*) *2*, and cell differentiation. Tha latter is achieved through its effect on activating genes such as *Aim-1*, *Dct*, *Tyrosinase related protein 1* (*TYRP1*), *Tyrosinase* (*TYR*), *Mart1*, *Silver/Pmel17 and Mc1r* [167]. In zebrafish embryos, *nacre* is a counterpart of *mitf*. This molecule is responsible for the production of a transcription factor which controls the transcription of genes involved in the pigmentation process, like *trp-1*. The referred regulation is achieved through WNT signaling [108]. Considering the major contribution of *Mitf* in melanocyte development, it has to be mentioned that additional transcription factors are required for the full melanocyte differentiation. Specifically, in mice *Sox10* is necessary for the expression of *Dct/Trp2*, whose transcription product is required for the proper synthesis of eumelanin. Eumelanin is the basic type of melanin, produced by tyrosine through the process of melanogenesis. The interaction of *Sox10* with

Dct/Trp2 can be both direct and indirect, through the mediation of *Mitf* [91,168]. However, recent examination of this pathway in zebrafish proposes a different scenario, indicating that *sox10* might actually have a negative effect on the expression of melanogenic enzymes. In this hypothesis *mitf* acts as a repressor of *sox10* [87]. In Zebrafish the distinct role of *sox10* is the regulation of *mitf* in a linear way, without affecting the transcription of other genes. The direct correlation between *SOX10* and the activation of *MITF* can be noted in human crest cells too [86]. It can be easily concluded that mutations in *SOX10* are directly associated with dysregulated melanocytic differentiation, leading in neuroectodermal tumors, like melanoma [4]. *Pax3* is another important gene involved in the pigmentation procedure in ascidian embryos. Firstly it increases the survival rate of melanocyte precursors and secondly it activates *Mitf* promoter along with *Sox10*. However, the hypothesis of the immediate control of *Mitf* needs further investigation [169,170]. On the other hand, an antagonist in the promotion of melanocyte differentiation induced by *mitf*, is *foxd3*, which restrains its transcription. As a result, it prevents crest cells from evolving into melanocytes in zebrafish [171]. An additional gene that is involved in the process of pigmentation during the early stages of mice embryogenesis is *Mef2c*, along with its transcriptional product MEF2C. MEF2C's loss of function causes defects in the process of melanogenesis, due to a decrease in the number of differentiated melanocytes. It is responsible for defects in the gut innervation as well [172]. MEF2 protein family plays a significant role in governing the development of the brain, lymphocytes, skeleton, and cardiovascular system in many vertebrates including mice and Drosophila. However, in Drosophila there is only one *Mef2* gene, instead of a protein family, which is pivotal for the proper melanogenesis [173]. In mouse embryos, *Mefc2c* enhancer contains three functional sites that bind to SOX proteins as well as a crucial MEF2 site. This indicates that *Mefc2c* is directly regulated and dependent on the SOX10 and MEF2 protein family. The transcription factor MEF2C, encoded by the *Mef2c* gene, has been proven to preserve a differentiating phenotype in melanoblasts. Cosequently, it is on charge of the proper amount of melanoblasts [172].

The major molecular signaling pathways that take part in the procedure of melanocyte formation include WNT, KIT proto-oncogene (KIT) and EDNRB. To begin with, canonical Wnt/β-catenin signaling cascade is necessary for the stimulation of NC and for the initiation of melanocytic development. By binding to its transmembrane receptor Frizzled, Wnt activates a series of transcription factors whose role is to regulate the transcription of certain genes inside the nucleus in Xenopus [174]. WNT signaling acts through direct activation of β catenin and it has been proven that, in Zebrafish embryos, elevated levels of β-catenin stimulate melanoblast development. At the same time, increased β-catenin diminishing levels diminish the formation of neurons and glia [175]. Both in mouse and chick populations, *Wnt1* and *Wnt3*, (*WNT1* and *WNT3* respectively), are prerequisites for proliferation and differentiation of melanocytes, thus indicating the crucial role of WNT/β-catenin in the formation of pigmented cells. In human embryos, β-catenin/TCF signaling cascade directly regulates the capacity of *MITF* to activate itself [176]. Concerning KIT signaling, it acts synergistically with *MITF* and determines the extent of the proliferation of melanocyte precursors. However, the precise mechanism remains incompletely understood [88,89,177]. *KIT* is usually detected in melanocytes, where it is necessary for melanoblast migration. Additionally, it is present in cell populations like blood cells and primordial germ cells [178–180]. Pigmentation defects can be caused by mutations in genes such as *KIT*, causing Piebaldism, an autosomal dominant disease. Lastly, binding of EDN3 to its receptor EDNRB is considered to be crucial for the migration of melanocyte and enteric neuroblast progenitors in mice, before cell differentiation takes place [90,181]. This cascade encloses the induction of pathways like MAPK, CAMK2 and PKC [182].

2.4 Enteric

The progenitors of ENS are the vagal and sacral NC. Cells from these regions start migrating, multiplying and finally they give rise to both neurons and glial cell products in the developing embryonic gut. ENS consists of operational neural networks consisting of various types of neurons and glia, structured into two linked clusters of ganglia: the myenteric and submucosal plexuses within the gut. Vagal NC, situated between one and seven somites, is responsible for forming the vast majority of the ENS, with its cells colonising the digestive tube in a 'head to tail' direction. This cell population is divided into two subtypes; one extending from somites level one through 3 and the second lying between somites four through seven. The first subtype is separated in two smaller groups, the first of which migrates in a dorsolateral direction beneath the ectoderm to populate the pharyngeal arches and the cardiac outflow tract, while the second migrates ventrally and gives birth to the dorsal root and sympathetic ganglia as well as forming part of the ENS by invading into the initial part of the foregut [183]. Crest cells from somite level four through seven move ventrally and end up colonising the gut [184]. According to other subdivisions, in chicken embryos NCCs in the surroundings of somites one and two generate the circumpharyngeal crest as well as precursor cells for Schwann cells. These precursors then populate the vagus nerve, subsequently directing their migration into the esophagus and stomach. They establish themselves along nerves by detecting axonal Nrg1 via the tyrosine kinase receptor ErbB3. *ErbB3* is, also, required for the development of the sympathetic nervous system in mouse embryos [78,97]. On the other hand, crest cells positioned near somites three through seven, take part in the formation of the sympa-

thetic ganglion chain and invade the whole digestive tube [185]. Sacral crest provides approximately 20% of the neurons found in the descending colon and rectum.

To begin, retinoic acid (RA) plays a crucial role in the differentiation of NCCs into enteric lineage. RA is secreted by the paraxial mesoderm in the space around somites in quail embryos and interacts with its receptors RARα, RARα2, RARγ that are located on NCCs surface [186]. This interaction results in the activation of the receptor tyrosine kinase RET, which is required for the proper formation of the ENS [187]. Apart from its significance in cell survival and migration as mentioned before, *Ret* is necessary for the survival of post migratory enteric neurons in rodent embryos [188]. Another molecule that has a crucial role in the migration of ENCCs is tenascinC. In chicken embryos, tenascinC (TNC), an extracellular matrix glycoprotein, is produced and secreted by migrating vagal-derived ENCCs and by the gut environment. Crest cells and the gut mesenchyme secrete different isoforms of TNC [189]. The presence of vagal crest-derived ENCCs determines the expression of TNC in the inner submucosal layers of the colon barrier, whereas TNC expression in the outer muscular tissue layer is not influenced by ENCCs. This is because the external gut wall can independently generate TNC, potentially through smooth muscle cells [190]. One of its functions is to promote ENCCs' migration into their final destination, as it antagonizes adhesiveness of NCCs [191], and restrains their integrin-mediated adhesion to fibronectin [192]. It has, though, to be mentioned that contradictory evidence about the function of TNC have been found. In experiment [193], TNC is supposed to block the migration of enteric crest cells, a result explained probably by differences in the embryos that were used, in this case mice, the quantity of the chemical molecules, as well as the substrates that were used. In mice embryos, TNC's hindrance of migration was overcome using β1 integrins. These molecules proved to enhance the migration rates, that in this animal model are based on fibronectin. As the formation of the ENS proceeds, mobility of crest cells is reduced at the region of the cecum. This gives them the ability to invade the colon as independent cells and not in cell groups [189].

Netrins are a significant laminin related protein family, generated by epithelial cells located in the gut. They have been demonstrated as significant participants in the development of ENS, contributing in the surveillance and migration of ENCCs. To begin with, they are being expressed in the developing intestine and pancreas in mouse and chick embryos. NETRIN-1 is a member of the family that acts as an attractive source for ENCCs and fosters not only the growth but also viability of cells originating from enteric crest. It is also responsible for the axonal growth of neurites. Specifically in order to enhance neurite extension, NETRIN-1 necessitates co-factors that are generated by cells within the bowel wall. However, these co-factors do not originate from the crest. Netrins, also, stimulate the inward migration of neurons from the myenteric to the submucosal region, with mice missing these protein molecules, lacking the submucosal as well [194]. Another protein family produced by the epithelial cells in mouse embryos is hedgehog (Hh) family, crucial for controlling the accumulation and specialization of the mesenchyme [195,196]. Hedgehog proteins, including sonic hedgehog (SHH) and indian hedgehog (IHH), are signaling molecules which are secreted by the enteric epithelium from the initial phases of intestinal organ formation. They directly affect BMP4 production, which is pivotal for the proper ECM formation and prevents the development of smooth muscle under the enteric epithelium. Moreover, this group of proteins is involved in the activation of *Foxf1* and *Foxf2*, in the mesenchymal cells (forkhead transcription factors), which are crucial for the proper production and formation of the ECM (especially *Foxf2*). FOXF proteins are responsible for the induction of BMP4, which in turn inhibits the transcription of *Wnt5a*. In mutant mice with loss of *Foxf2* alleles, this imbalance in signaling molecules leads to a decrease in collagen synthesis and finally in general ECM disintegration. Such deficiency of the ECM disrupts interactions between confinement molecules and as a result, epithelial cells of the intestinal villi lose their normal polarisation and become round with centrally located nuclei. However, cells remain viable and present resilience in apoptosis due to increased WNT signaling and β-catenin levels. In mouse fetuses lacking *Foxf*, the wall of the distant part of the colon is unnaturally thin and expanded, a situation described as megacolon, which also presents atresia and is aganglionic [94]. Another role of BMP4 is to hinder the precocious differentiation of ENCCs. BMPs seem, also, to influence the balance between neurons and glial cells in the mouse gut. For instance, blocking BMP signaling using NOGGIN results in a rise in the overall number of neurons [95], while reducing the density of glial cells. Conversely, an overexpression of BMP increases the ratio of glial cells to neurons [184].

Moreover, EDNRB signaling encourages the growth of ENCCs while preventing their transformation into neurons [73,184]. This effectively keeps them in an undifferentiated and multiplying condition which comes in contrast with GDNF signaling. The latter, drives progenitor cells to differentiate into neurons [163]. ENDRB signaling pathway is not necessary until the migration stream of NCCs invades the region of the cecum. In the balance between neurons and glial cells in mice, *Ezh2* is also involved. *Ezh2*'s expression is increased in ENCCs committed to the glial lineage, where it functions as a transcriptional activator. On the other hand, it is reduced in cells that are going to differentiate into neurons, where it serves the role of transcriptional repressor [197]. *Ezh2* is regulated by Kinesin family member 7 (*Kif7*) [96]. Transcriptional factors detected in glial cell progenitors are Sox10, Erbb3, Fabp7,

and Plp1, while those participating in the determination of the neuronal fate include Elavl4, Tubb3, Phox2b and Ret [197,198].

The procedure of the terminal differentiation of ENCCs encloses different molecules. In particular, ASCL1 and HAND2 are two transcription factors that are also involved in the process of enteric nervous system formation, especially in their terminal differentiation [89]. Specifically, *Ascl1* is present in all progenitors of enteric neuronal subtypes in mouse and human embryos (*ASCL1*). It is required for proper neurogenesis, with its loss being associated with delayed formed and atrophic neurons. But even in its absence, migration rates are not affected. Neuronal development in mutant embryos can be saved by increased expression of *Ngn2*, a proneural gene. However, *Ngn2* cannot replace *Ascl1*'s function in determining the formation of particular types of the enteric neurons. Consequently, *Ascl1* is a necessary transcription factor in the normal differentiation procedure [92]. Another gene involved in the terminal differentiation of ENCCs is *Hand2*. It is a prerequisite for the differentiation of NCCs into enteric neurons in mice and is present in cells that are undergoing development as both neurons and glial cells. In mice mutants that do not express *Hand2*, crest-derived precursors usually populate the *Hand2*-deficient gut, and its presence is not necessary for the initial activation of *Phox2B*, *Ret* or *Sox10*. However, its expression is essential for the final stage of differentiation of neurons located in the gut [93]. Additionally, in mutant mice lacking *Hand2*, notable reductions are observed in the counts of cholinergic and nitrergic neurons. Nitric oxide synthetase (NOS), which is necessary for the formation of NO, is missing in these mutant embryos, demonstrating its downstream dependance on *Hand2*. NOS offers guidance for the migration of particular groups of cells [199]. Since cells producing two crucial neurotransmitters in the enteric nervous system, nitric oxide (NO) and acetylcholine (ACh), tend to develop early in the developmental process, the substantial decrease in both of these cell types in the progenitors of neural endopeptidase cells implies that neurotransmission would likely be adversely impacted.

3. Pathological Alterations Attributed to NC-Related Gene Changes

When NC genes are altered, NCCs' migration is disrupted and characteristic phenotypic changes occur. These phenotypes are thoroughly studied using various animal models that help elucidate the complexity of gene pathways and reveal the distinct phenotypic alterations that occur when these pathways are compromised. Zebrafish, Xenopus, avian, and mouse embryos each offer unique insights into the complexities of NC migration and differentiation. Xenopus, an adaptable vertebrate model, has been instrumental in studying various neurocristopathies through diverse experimental methodologies. Its large eggs allow for early-stage injections, facilitating accessibility throughout embryonic development. Additionally, the transparency of Xenopus tadpoles in later stages enables imaging akin to zebrafish larvae, thus enabling the monitoring of differentiated cells originating from the neural crest [200].

The avian embryo has been pivotal in elucidating cell migration and histogenesis since the inception of the quail-chick marking system in 1969. Through this method, chimeras generated by replacing specific embryonic regions of one species with those of another enable the investigation of the behavior and destiny of grafted NC cells. By substituting fragments of the neural fold or tube between chick and quail embryos along the neural axis, migration pathways and fates of neural crest cells exiting at different levels of the neural tube can be discerned [201]. Although mice models are valuable for studying NC pathways in mammalian species, they have limitations. For instance, null mice lacking key NC genes sometimes do not exhibit a failure of neural crest cell induction similar to non-mammalian organisms [202]. Nevertheless, the applications of all these animal models contribute to deciphering the "master genes" of neural crest cells and their morphogenetic fate.

The pathological changes due to NC-related gene alterations that are observed in these animal models are categorized under the umbrella term "Neurocristopathies", which encompasses a broad spectrum of disorders arising from issues during migration and differentiation of NCCs. Mutations in *EphA2* led to abnormal migration of cranial NC and loss of the distinction between third and fourth arch streams [28]. Regarding FGF signaling, mice mutants with Fgfr2 deficiency display reduced osteoblast differentiation [51]. FGFR2 is also implicated in syndromic craniosynostosis [203]. Multiple craniofacial defects with the most prominent of them being agenesis of frontal bones and absence of parietal and interparietal ossification is exhibited in *Dlx5* mutant embryos, with this gene being involved in the pathogenesis of frontonasal dysplasia. Absence of Twist1 function leads to anomalies in the cranial mesenchyme, hindrance in the closure of the neural tube in the cranial area, and various other anomalies, including Saethre–Chotzen syndrome [204]. The temporary deactivation of Hoxa2 from the stage before migration to the later stages after migration consistently results in the complete alteration of skeletal structures in the second arch to resemble those of the first arch, as seen in Hoxa2-deficient mutants [205]. Embryos with *Dlx1/2* mutations have impaired development of the upper jaw and the hinge region, while in brain development *Dlx1/2* mutations are linked to abnormal striatal subventricular zone formation and differentiation [63,206]. Loss of *Dlx5* leads in proximal lower jaw anomalies, profound deformities in the vestibular system, postponed ossification in the cranial vault, irregular bone development and abnormalities in the hinge region [55] Tricho-Dento-Osseous syndrome is likely connected to *Dlx5* mutations. The simultaneous inactivation of both

Dlx5 and *Dlx6* at the same time results in a lower jaw that mirrors the upper jaw [207,208].

When it comes to placode formation, mutated mice with loss of TGFβ signaling exhibit eyes of smaller size with a deficiency in the endothelial layer and the ciliary body. Additionally, there is an accumulation of mesenchyme between the lens and retina, an enlargement of the vitreous, and disruption in retinal patterning [108]. As *pax6* is responsible for the appropriate guidance of the two NC streams that enter the optic cup, as well as for the expression of guidance molecules that aid this procedure, its loss can lead to serious anterior segment abnormalities [111]. Its loss is connected to Peter's anomaly and congenital aniridia [209]. As *Dlx* genes are involved in craniofacial development, their mutations also affect ear morphogenesis. Mutations in *Dlx5/6*, the incus is up to a point duplicated along the dorsoventral axis, while regarding the PA2, the stapes lacks its foramen [208]. *Hoxa2*-null mutants exhibit duplicated incus and maleus in the PA2, as the PA2 mirrors the structures of PA1 [61,62].

Various heart abnormalities are also observed. *Tbx1* deactivation leads to diminished NC cell presence in the heart and the maturation and differentiation of cardiac NCCs is hindered, due to a decrease in MAPK signaling and an early activation of BMP signaling [65]. *Tbx1* mutations are linked to Di George syndrome, CHARGE syndrome as well as Velocardiofacial syndrome [65,210]. Mouse embryos lacking either Sema3C or PlexinA2, are not able to properly arrange NCCs in the cardiac outflow tract [69,211]. Moreover, the absence of Semaphorin 3C leads to the disruption of the aortic arches' morphogenesis, while neuropilin containing receptors interact with vascular endothelial growth factor (VEGF) signaling [3,212]. *BMP1* inactivation results in the inability to separate the outflow tract and a lack of proper development of smooth muscle in the heart. Similarly, impairing BMP receptor 1A in NCCs also leads to defects in the outflow tract [131]. Loss of *SMAD4* results in several cardiac defects and extensive cell death in cardiac NCCs and NCCs populating the pharyngeal arches [213,214], while its disruption causes cardiac hypertrophy [215]. Loss of *Alk2* led to impaired cardiac NC migration and several cardiac and craniofacial defects [71,216]. Loss of *Prdm6* in cardiac NCCs leads to a disruption in the pre-EMT stage, causing NCCs to stay in the dorsal neural tube. Moreover, loss of Prdm6 in mice can result in thinner myocardial walls and subsequent heart failure [72].

Loss of *Mitf or Sox10* leads to pigmentation defects in mice and both genes are implicated in the pathogenesis of Waardenburg syndrome [86]. Disabling *Mef2c* within mice NC leads to diminished activation of genes associated with melanocytes during their development, causing a notable decrease in. This occurs as a consequence of impaired cellular differentiation and a decreased number of melanocytes [172]. *Alk3* deletion in embryos does not influence migration of neural progenitors towards the dorsal aorta but it weakens their ability to form aggregates, leading to cardiac abnormalities and high fatality rate. The absence of *Phox2b*, which is connected to Hirschprung disease, might explain the absence of sympathetic nervous system (SNS) precursors in *Alk3* knockout embryos [217].

4. Conclusions and Perspectives

In the current study, we attempted to organize specifier genes according to location and individual cell type basis with extension to related dysmorphogenesis in animal models. The NC contributes to craniofacial and placode development, with HOX family genes' and DLX family genes' guidance being the most prominent. In cardiac development, *TBX2/3, PAX3* and *PRDM6* are crucial, while in enteric development, *ASCL1, HAND2* and *RET* are key genes. When it comes to distinct cell types, neural-crest derived neuronal lineages are characterized by NGN1, NRP1/2, PAX3, SOX10 and C-RET expression. *KIT, MITF* and *SOX10* have important roles in melanocytes formation, SOX family genes are vital for chondrocyte fates. Additionally, we aimed to illustrate distinct genetic alterations that result in phenotypic examples across various species. Through such examination, a deep understanding of the fundamental processes involved in abnormal morphogenesis is acquired. Nonetheless, more systematic approaches should be implemented to achieve such a result. Understanding the intricate processes involved in NC cell migration and differentiation requires a roadmap of molecular cues and genetic drivers [218,219]. In this pursuit, animal models serve as essential tools for unraveling complex gene pathways.

The multipotency of NCCs presents intriguing avenues for research and potential medical applications. NCCs use can serve as a mean to tissue and organ repair and regeneration while potentionally aiding in the treatment of congenital forms of NC-related diseases [220,221]. Loss-of-function techniques, namely dominant-negative constructs, antisense morpholinos, and RNA interference (RNAi) have been preferred methods for transiently disrupting neural crest development in chick, Xenopus, and Zebrafish embryos. Miscellaneous technologies such as Cre/loxP, TALENs, Flp/FRT and newer ones like CRISPR-Cas9 are widely employed in generating knockout animals. However, challenges persist in NCC research, as these methods alone may not fully elucidate the underlying gene networks and interactions [222]. Moreover, variability in existing NC induction models poses further complications. Contemporary technologies, e.g., next-generation sequencing (NGS), proteomics and transcriptomics, may offer an alternative view into congenital disease progression and mechanisms of gene interactions [223]. Moreover, the knowledge aquired will accelerate the rise of "personalized medicine" and "precision medicine" [221]. Through these promising approaches, early diagnosis can be achieved and

specialized interventions catered to each patient can be designed, producing individualized therapeutic solutions.

Abbreviations

AAA, abdominal aortic aneurysm; ACH, acetylocholine; ALX, ALX homeobox; ASCL, achaete-scute family bHLH transcription factor; BARX, BARX homeobox; BMP, bone morphogenetic protein; BMPR1B, bone morphogenetic protein receptor 1 beta; CDK, cyclin-dependent kinase; CX, connexin; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C motif chemokine receptor 4; DLX, distal-less homeobox; DRG, dorsal root ganglia; DV, dorsal-ventral; ECM, extracellular matrix; EDNR, endothelin receptor; EMT, epithelial mesenchymal transition; ENCC, enteric neural crest cell; ENS, enteric nervous system; EPH, ephrin; ERBB4, erb-b2 receptor tyrosine kinase 4; ETS, ETS proto-oncogene; FGF, fibroblast growth factor; FLRT, fibronectin leucine-rich transmembrane; FOX, forkhead box; GBX, gastrulation brain homeobox; GDNF, glial cell derived neurotrophic factor; GSC, goosecoid; HAND, heart and neural crest derivatives; HH, hedgehog; HOX, homeobox; IHH, Indian hedgehog; KIT, KIT proto-oncogene; LHX, LIM homeobox; LIFR, LIF receptor subunit alpha; MITF, melanocyte inducing transcription factor; MMP, matrix metalloproteinase; MPNST, malignant peripheral nerve sheath tumor; MSX, MSH homeobox; MYH, myosin heavy chain; MYL, myosin light chain; NC, neural crest; NCC, neural crest cell; NEP, neuroepithelial; NF, neurofibromin; NO, nitric oxide; NOS, nitric oxide synthetase; NGN1/2, neurogenin-1/2; NRG, neuregulin; NRP, neuropilin; PA, pharyngeal arch; PAX, paired box; PHOX, paired like homeobox; PITX, paired like homeodomain; PNS, peripheral nervous system; PRDM, PR/SET domain; RA, retinoic acid; ROBO, roundabout; R3/5, rhombomere 3/5; SA, sympathoadrenal; SDF, stromal cell derived factor; SEMA, semaphorin; SHH, sonic hedgehog; SIP, sheath interacting protein; SMAD, SMAD family member; SNAP, synaptosome associated protein; SOX, SRY-box transcription factor; TAGLN, transgelin; TBX, T-box transcription factor; TGFBR2, TGF receptor type II; TH, tyrosine hydrosylase; TNC, tenascinC; TFAP, transcription factor AP; TGF, transforming growth factor; TYR, tyrosinase; TYRP, tyrosinase related protein 1; TWIST, twist family bHLH transcription factor; vSMC, vascular smooth muscle cell; ZIC1, ZIC family member 1.

Author Contributions

SAK and DC collected, analysed and reviewed the information, wrote and edited the manuscript; ID reviewed and assisted in the analysis; SG, MEM and SM provided advice on the review; PT conceptualized and designed the review. All authors contributed to editorial changes in the manuscript. All authors contributed to the drawing of charts and figures. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest. Given his role as Guest Editor, Paschalis Theotokis had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Margarita M Ivanova and Viviana di Giacomo.

References

[1] Shyamala K, Yanduri S, Girish HC, Murgod S. Neural crest: The fourth germ layer. Journal of Oral and Maxillofacial Pathology: JOMFP. 2015; 19: 221–229.

[2] Achilleos A, Trainor PA. Neural crest stem cells: discovery, properties and potential for therapy. Cell Research. 2012; 22: 288–304.

[3] Stoller JZ, Epstein JA. Cardiac neural crest. Seminars in Cell & Developmental Biology. 2005; 16: 704–715.

[4] Hou L, Pavan WJ. Transcriptional and signaling regulation in neural crest stem cell-derived melanocyte development: do all roads lead to Mitf? Cell Research. 2008; 18: 1163–1176.

[5] Miletich I, Sharpe PT. Neural crest contribution to mammalian tooth formation. Birth Defects Research. Part C, Embryo Today: Reviews. 2004; 72: 200–212.

[6] Minoux M, Rijli FM. Molecular mechanisms of cranial neural crest cell migration and patterning in craniofacial development. Development. 2010; 137: 2605–2621.

[7] Vega-Lopez GA, Cerrizuela S, Aybar MJ. Trunk neural crest cells: formation, migration and beyond. The International Journal of Developmental Biology. 2017; 61: 5–15.

[8] Dermitzakis I, Manthou ME, Meditskou S, Tremblay MÈ, Petratos S, Zoupi L, *et al*. Origin and Emergence of Microglia in the CNS-An Interesting (Hi)story of an Eccentric Cell. Current Issues in Molecular Biology. 2023; 45: 2609–2628.

[9] Dermitzakis I, Theotokis P, Evangelidis P, Delilampou E, Evangelidis N, Chatzisavvidou A, *et al*. CNS Border-Associated Macrophages: Ontogeny and Potential Implication in Disease. Current Issues in Molecular Biology. 2023; 45: 4285–4300.

[10] Theotokis P, Zoupi L, Tremblay MÈ, Zhao JW. Editorial: Molecular and cellular interactions of myelin in neurodevelopmental & neurodegenerative disorders. Frontiers in Cellular Neuroscience. 2024; 17: 1359184.

[11] Taneyhill LA, Schiffmacher AT. Cadherin dynamics during neural crest cell ontogeny. Progress in Molecular Biology and Translational Science. 2013; 116: 291–315.

[12] Couly GF, Coltey PM, Le Douarin NM. The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Development. 1993; 117: 409–429.

[13] Théveneau E, Duband JL, Altabef M. Ets-1 confers cranial features on neural crest delamination. PloS One. 2007; 2: e1142.
[14] Simões-Costa MS, McKeown SJ, Tan-Cabugao J, Sauka-Spengler T, Bronner ME. Dynamic and differential regulation of stem cell factor FoxD3 in the neural crest is Encrypted in the genome. PLoS Genetics. 2012; 8: e1003142.
[15] Betancur P, Bronner-Fraser M, Sauka-Spengler T. Assembling neural crest regulatory circuits into a gene regulatory network. Annual Review of Cell and Developmental Biology. 2010; 26: 581–603.
[16] Rogers CD, Saxena A, Bronner ME. Sip1 mediates an E-cadherin-to-N-cadherin switch during cranial neural crest EMT. The Journal of Cell Biology. 2013; 203: 835–847.
[17] Yasumi T, Inoue M, Maruhashi M, Kamachi Y, Higashi Y, Kondoh H, *et al*. Regulation of trunk neural crest delamination by δEF1 and Sip1 in the chicken embryo. Development, Growth & Differentiation. 2016; 58: 205–214.
[18] Lumsden A, Sprawson N, Graham A. Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. Development. 1991; 113: 1281–1291.
[19] Smith A, Robinson V, Patel K, Wilkinson DG. The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. Current Biology: CB. 1997; 7: 561–570.
[20] Golding JP, Trainor P, Krumlauf R, Gassmann M. Defects in pathfinding by cranial neural crest cells in mice lacking the neuregulin receptor ErbB4. Nature Cell Biology. 2000; 2: 103–109.
[21] Golding JP, Sobieszczuk D, Dixon M, Coles E, Christiansen J, Wilkinson D, *et al*. Roles of erbB4, rhombomere-specific, and rhombomere-independent cues in maintaining neural crest-free zones in the embryonic head. Developmental Biology. 2004; 266: 361–372.
[22] Yu HH, Moens CB. Semaphorin signaling guides cranial neural crest cell migration in zebrafish. Developmental Biology. 2005; 280: 373–385.
[23] Osborne NJ, Begbie J, Chilton JK, Schmidt H, Eickholt BJ. Semaphorin/neuropilin signaling influences the positioning of migratory neural crest cells within the hindbrain region of the chick. Developmental Dynamics. 2005; 232: 939–949.
[24] Gammill LS, Gonzalez C, Bronner-Fraser M. Neuropilin 2/semaphorin 3F signaling is essential for cranial neural crest migration and trigeminal ganglion condensation. Developmental Neurobiology. 2007; 67: 47–56.
[25] Mellott DO, Burke RD. Divergent roles for Eph and ephrin in avian cranial neural crest. BMC Developmental Biology. 2008; 8: 56.
[26] Schwarz Q, Waimey KE, Golding M, Takamatsu H, Kumanogoh A, Fujisawa H, *et al*. Plexin A3 and plexin A4 convey semaphorin signals during facial nerve development. Developmental Biology. 2008; 324: 1–9.
[27] Scales JB, Winning RS, Renaud CS, Shea LJ, Sargent TD. Novel members of the eph receptor tyrosine kinase subfamily expressed during Xenopus development. Oncogene. 1995; 11: 1745–1752.
[28] Helbling PM, Tran CT, Brändli AW. Requirement for EphA receptor signaling in the segregation of Xenopus third and fourth arch neural crest cells. Mechanisms of Development. 1998; 78: 63–79.
[29] Mori-Akiyama Y, Akiyama H, Rowitch DH, de Crombrugghe B. Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100: 9360–9365.
[30] Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. Nature Genetics. 1999; 22: 85–89.
[31] Han Y, Lefebvre V. L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. Molecular and Cellular Biology. 2008; 28: 4999–5013.
[32] Liu H, Lan Y, Xu J, Chang CF, Brugmann SA, Jiang R. Odd-skipped related-1 controls neural crest chondrogenesis during tongue development. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110: 18555–18560.
[33] Akiyama H, Lyons JP, Mori-Akiyama Y, Yang X, Zhang R, Zhang Z, *et al*. Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Genes & Development. 2004; 18: 1072–1087.
[34] Soeda T, Deng JM, de Crombrugghe B, Behringer RR, Nakamura T, Akiyama H. Sox9-expressing precursors are the cellular origin of the cruciate ligament of the knee joint and the limb tendons. Genesis (New York, N.Y.: 2000). 2010; 48: 635–644.
[35] Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, *et al*. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell. 2002; 108: 17–29.
[36] Ferguson GB, Van Handel B, Bay M, Fiziev P, Org T, Lee S, *et al*. Mapping molecular landmarks of human skeletal ontogeny and pluripotent stem cell-derived articular chondrocytes. Nature Communications. 2018; 9: 3634.
[37] Ridgeway AG, Skerjanc IS. Pax3 is essential for skeletal myogenesis and the expression of Six1 and Eya2. The Journal of Biological Chemistry. 2001; 276: 19033–19039.
[38] Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. Cell. 2000; 102: 777–786.
[39] Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell. 1987; 51: 987–1000.
[40] Braun T, Rudnicki MA, Arnold HH, Jaenisch R. Targeted inactivation of the muscle regulatory gene Myf-5 results in abnormal rib development and perinatal death. Cell. 1992; 71: 369–382.
[41] Cameron TL, Belluoccio D, Farlie PG, Brachvogel B, Bateman JF. Global comparative transcriptome analysis of cartilage formation in vivo. BMC Developmental Biology. 2009; 9: 20.
[42] Haq R, Fisher DE. Biology and clinical relevance of the microphthalmia family of transcription factors in human cancer. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2011; 29: 3474–3482.
[43] Goldring MB, Tsuchimochi K, Ijiri K. The control of chondrogenesis. Journal of Cellular Biochemistry. 2006; 97: 33–44.
[44] Zeng Q, Li W, Lu D, Wu Z, Duan H, Luo Y, *et al*. CD146, an epithelial-mesenchymal transition inducer, is associated with triple-negative breast cancer. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 1127–1132.
[45] Swart GWM. Activated leukocyte cell adhesion molecule (CD166/ALCAM): developmental and mechanistic aspects of cell clustering and cell migration. European Journal of Cell Biology. 2002; 81: 313–321.
[46] Lunter PC, van Kilsdonk JWJ, van Beek H, Cornelissen IMHA, Bergers M, Willems PHGM, *et al*. Activated leukocyte cell adhesion molecule (ALCAM/CD166/MEMD), a novel actor in invasive growth, controls matrix metalloproteinase activity. Cancer Research. 2005; 65: 8801–8808.
[47] Wu L, Bluguermann C, Kyupelyan L, Latour B, Gonzalez S, Shah S, *et al*. Human developmental chondrogenesis as a basis for engineering chondrocytes from pluripotent stem cells. Stem Cell Reports. 2013; 1: 575–589.
[48] Jiang X, Iseki S, Maxson RE, Sucov HM, Morriss-Kay GM. Tis-

sue origins and interactions in the mammalian skull vault. Developmental Biology. 2002; 241: 106–116.
[49] Sasaki T, Ito Y, Bringas P, Jr, Chou S, Urata MM, Slavkin H, *et al*. TGFbeta-mediated FGF signaling is crucial for regulating cranial neural crest cell proliferation during frontal bone development. Development. 2006; 133: 371–381.
[50] Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, *et al*. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nature Genetics. 2005; 37: 275–281.
[51] Yu K, Xu J, Liu Z, Sosic D, Shao J, Olson EN, *et al*. Conditional inactivation of FGF receptor 2 reveals an essential role for FGF signaling in the regulation of osteoblast function and bone growth. Development. 2003; 130: 3063–3074.
[52] Wrana JL, Attisano L. MAD-related proteins in TGF-beta signalling. Trends in Genetics: TIG. 1996; 12: 493–496.
[53] Wrana JL, Attisano L, Cárcamo J, Zentella A, Doody J, Laiho M, *et al*. TGF beta signals through a heteromeric protein kinase receptor complex. Cell. 1992; 71: 1003–1014.
[54] Zhang Y, Feng X, We R, Derynck R. Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. Nature. 1996; 383: 168–172.
[55] Depew MJ, Liu JK, Long JE, Presley R, Meneses JJ, Pedersen RA, *et al*. Dlx5 regulates regional development of the branchial arches and sensory capsules. Development. 1999; 126: 3831–3846.
[56] Tadic T, Dodig M, Erceg I, Marijanovic I, Mina M, Kalajzic Z, *et al*. Overexpression of Dlx5 in chicken calvarial cells accelerates osteoblastic differentiation. Journal of Bone and Mineral Research: the Official Journal of the American Society for Bone and Mineral Research. 2002; 17: 1008–1014.
[57] Jabs EW. A TWIST in the fate of human osteoblasts identifies signaling molecules involved in skull development. The Journal of Clinical Investigation. 2001; 107: 1075–1077.
[58] Maretto S, Müller PS, Aricescu AR, Cho KWY, Bikoff EK, Robertson EJ. Ventral closure, headfold fusion and definitive endoderm migration defects in mouse embryos lacking the fibronectin leucine-rich transmembrane protein FLRT3. Developmental Biology. 2008; 318: 184–193.
[59] Wei K, Xu Y, Tse H, Manolson MF, Gong SG. Mouse FLRT2 interacts with the extracellular and intracellular regions of FGFR2. Journal of Dental Research. 2011; 90: 1234–1239.
[60] Gong SG, Mai S, Chung K, Wei K. Flrt2 and Flrt3 have overlapping and non-overlapping expression during craniofacial development. Gene Expression Patterns: GEP. 2009; 9: 497–502.
[61] Rijli FM, Mark M, Lakkaraju S, Dierich A, Dollé P, Chambon P. A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. Cell. 1993; 75: 1333–1349.
[62] Gendron-Maguire M, Mallo M, Zhang M, Gridley T. Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. Cell. 1993; 75: 1317–1331.
[63] Depew MJ, Simpson CA, Morasso M, Rubenstein JLR. Reassessing the Dlx code: the genetic regulation of branchial arch skeletal pattern and development. Journal of Anatomy. 2005; 207: 501–561.
[64] Feng J, Bi C, Clark BS, Mady R, Shah P, Kohtz JD. The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes & Development. 2006; 20: 1470–1484.
[65] De Bono C, Liu Y, Ferrena A, Valentine A, Zheng D, Morrow BE. Single-cell transcriptomics uncovers a non-autonomous Tbx1-dependent genetic program controlling cardiac neural crest cell development. Nature Communications. 2023; 14: 1551.
[66] Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P. Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. The EMBO Journal. 1991; 10: 1135–1147.
[67] Conway SJ, Henderson DJ, Copp AJ. Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. Development. 1997; 124: 505–514.
[68] Foerst-Potts L, Sadler TW. Disruption of Msx-1 and Msx-2 reveals roles for these genes in craniofacial, eye, and axial development. Developmental Dynamics. 1997; 209: 70–84.
[69] Kirby ML, Hutson MR. Factors controlling cardiac neural crest cell migration. Cell Adhesion & Migration. 2010; 4: 609–621.
[70] Chen L, Fulcoli FG, Tang S, Baldini A. Tbx1 regulates proliferation and differentiation of multipotent heart progenitors. Circulation Research. 2009; 105: 842–851.
[71] Hong L, Li N, Gasque V, Mehta S, Ye L, Wu Y, *et al*. Prdm6 controls heart development by regulating neural crest cell differentiation and migration. JCI Insight. 2022; 7: e156046.
[72] Gewies A, Castineiras-Vilarino M, Ferch U, Jährling N, Heinrich K, Hoeckendorf U, *et al*. Prdm6 is essential for cardiovascular development in vivo. PloS One. 2013; 8: e81833.
[73] Wu JJ, Chen JX, Rothman TP, Gershon MD. Inhibition of in vitro enteric neuronal development by endothelin-3: mediation by endothelin B receptors. Development. 1999; 126: 1161–1173.
[74] Lang D, Chen F, Milewski R, Li J, Lu MM, Epstein JA. Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. The Journal of Clinical Investigation. 2000; 106: 963–971.
[75] Durbec PL, Larsson-Blomberg LB, Schuchardt A, Costantini F, Pachnis V. Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. Development. 1996; 122: 349–358.
[76] Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature. 1999; 399: 366–370.
[77] Blaugrund E, Pham TD, Tennyson VM, Lo L, Sommer L, Anderson DJ, *et al*. Distinct subpopulations of enteric neuronal progenitors defined by time of development, sympathoadrenal lineage markers and Mash-1-dependence. Development. 1996; 122: 309–320.
[78] Britsch S, Li L, Kirchhoff S, Theuring F, Brinkmann V, Birchmeier C, *et al*. The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. Genes & Development. 1998; 12: 1825–1836.
[79] Lumb R, Schwarz Q. Sympathoadrenal neural crest cells: the known, unknown and forgotten? Development, Growth & Differentiation. 2015; 57: 146–157.
[80] Zirlinger M, Lo L, McMahon J, McMahon AP, Anderson DJ. Transient expression of the bHLH factor neurogenin-2 marks a subpopulation of neural crest cells biased for a sensory but not a neuronal fate. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99: 8084–8089.
[81] Sommer L, Ma Q, Anderson DJ. neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. Molecular and Cellular Neurosciences. 1996; 8: 221–241.
[82] Ma Q, Kintner C, Anderson DJ. Identification of neurogenin, a vertebrate neuronal determination gene. Cell. 1996; 87: 43–52.
[83] Lumb R, Wiszniak S, Kabbara S, Scherer M, Harvey N, Schwarz Q. Neuropilins define distinct populations of neural crest cells. Neural Development. 2014; 9: 24.
[84] Schwarz Q, Maden CH, Vieira JM, Ruhrberg C. Neuropilin 1 signaling guides neural crest cells to coordinate pathway choice with cell specification. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106: 6164–

6169.
[85] Hou L, Arnheiter H, Pavan WJ. Interspecies difference in the regulation of melanocyte development by SOX10 and MITF. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103: 9081–9085.
[86] Watanabe A, Takeda K, Ploplis B, Tachibana M. Epistatic relationship between Waardenburg syndrome genes MITF and PAX3. Nature Genetics. 1998; 18: 283–286.
[87] Greenhill ER, Rocco A, Vibert L, Nikaido M, Kelsh RN. An iterative genetic and dynamical modelling approach identifies novel features of the gene regulatory network underlying melanocyte development. PLoS Genetics. 2011; 7: e1002265.
[88] Lister JA, Robertson CP, Lepage T, Johnson SL, Raible DW. nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. Development. 1999; 126: 3757–3767.
[89] Tsujimura T, Morii E, Nozaki M, Hashimoto K, Moriyama Y, Takebayashi K, *et al*. Involvement of transcription factor encoded by the mi locus in the expression of c-kit receptor tyrosine kinase in cultured mast cells of mice. Blood. 1996; 88: 1225–1233.
[90] Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, *et al*. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell. 1994; 79: 1267–1276.
[91] Aroca P, Solano F, Salinas C, García-Borrón JC, Lozano JA. Regulation of the final phase of mammalian melanogenesis. The role of dopachrome tautomerase and the ratio between 5,6-dihydroxyindole-2-carboxylic acid and 5,6-dihydroxyindole. European Journal of Biochemistry. 1992; 208: 155–163.
[92] Memic F, Knoflach V, Sadler R, Tegerstedt G, Sundström E, Guillemot F, *et al*. Ascl1 Is Required for the Development of Specific Neuronal Subtypes in the Enteric Nervous System. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2016; 36: 4339–4350.
[93] D'Autréaux F, Morikawa Y, Cserjesi P, Gershon MD. Hand2 is necessary for terminal differentiation of enteric neurons from crest-derived precursors but not for their migration into the gut or for formation of glia. Development. 2007; 134: 2237–2249.
[94] Ormestad M, Astorga J, Landgren H, Wang T, Johansson BR, Miura N, *et al*. Foxf1 and Foxf2 control murine gut development by limiting mesenchymal Wnt signaling and promoting extracellular matrix production. Development. 2006; 133: 833–843.
[95] Chalazonitis A, Pham TD, Li Z, Roman D, Guha U, Gomes W, *et al*. Bone morphogenetic protein regulation of enteric neuronal phenotypic diversity: relationship to timing of cell cycle exit. The Journal of Comparative Neurology. 2008; 509: 474–492.
[96] Lai FPL, Li Z, Zhou T, Leung AOW, Lau ST, Lui KNC, *et al*. Ciliary protein Kif7 regulates Gli and Ezh2 for initiating the neuronal differentiation of enteric neural crest cells during development. Science Advances. 2021; 7: eabf7472.
[97] Saito D, Takahashi Y. Sympatho-adrenal morphogenesis regulated by the dorsal aorta. Mechanisms of Development. 2015; 138 Pt 1: 2–7.
[98] Singh S, Groves AK. The molecular basis of craniofacial placode development. Wiley Interdisciplinary Reviews. Developmental Biology. 2016; 5: 363–376.
[99] Schlosser G. Making senses development of vertebrate cranial placodes. International Review of Cell and Molecular Biology. 2010; 283: 129–234.
[100] Theveneau E, Mayor R. Can mesenchymal cells undergo collective cell migration? The case of the neural crest. Cell Adhesion & Migration. 2011; 5: 490–498.
[101] Theveneau E, Marchant L, Kuriyama S, Gull M, Moepps B, Parsons M, *et al*. Collective chemotaxis requires contact-dependent cell polarity. Developmental Cell. 2010; 19: 39–53.
[102] Bajanca F, Gouignard N, Colle C, Parsons M, Mayor R, Theveneau E. In vivo topology converts competition for cell-matrix adhesion into directional migration. Nature Communications. 2019; 10: 1518.
[103] Barriga EH, Theveneau E. *In vivo* Neural Crest Cell Migration Is Controlled by "Mixotaxis". Frontiers in Physiology. 2020; 11: 586432.
[104] Garmon T, Wittling M, Nie S. MMP14 Regulates Cranial Neural Crest Epithelial-to-Mesenchymal Transition and Migration. Developmental Dynamics. 2018; 247: 1083–1092.
[105] Williams AL, Bohnsack BL. Neural crest derivatives in ocular development: discerning the eye of the storm. Birth Defects Research. Part C, Embryo Today: Reviews. 2015; 105: 87–95.
[106] Williams AL, Bohnsack BL. The Ocular Neural Crest: Specification, Migration, and Then What? Frontiers in Cell and Developmental Biology. 2020; 8: 595896.
[107] Langenberg T, Kahana A, Wszalek JA, Halloran MC. The eye organizes neural crest cell migration. Developmental Dynamics. 2008; 237: 1645–1652.
[108] Taneyhill LA, Bronner-Fraser M. Recycling signals in the neural crest. Journal of Biology. 2005; 4: 10.
[109] Ittner LM, Wurdak H, Schwerdtfeger K, Kunz T, Ille F, Leveen P, *et al*. Compound developmental eye disorders following inactivation of TGFbeta signaling in neural-crest stem cells. Journal of Biology. 2005; 4: 11.
[110] Gallik KL, Treffy RW, Nacke LM, Ahsan K, Rocha M, Green-Saxena A, *et al*. Neural crest and cancer: Divergent travelers on similar paths. Mechanisms of Development. 2017; 148: 89–99.
[111] Singhal D, Tripathy K. Oculo Auriculo Vertebral Spectrum. StatPearl: Treasure Island (FL). 2024.
[112] Halder G, Callaerts P, Gehring WJ. Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. Science (New York, N.Y.). 1995; 267: 1788–1792.
[113] Whitfield TT. Development of the inner ear. Current Opinion in Genetics & Development. 2015; 32: 112–118.
[114] Torres M, Giráldez F. The development of the vertebrate inner ear. Mechanisms of Development. 1998; 71: 5–21.
[115] Sandell L. Chapter 9 - Neural Crest Cells in Ear Development. In Trainor PA (ed.) Neural Crest Cells (pp. 167–87). Academic Press: Boston. 2014.
[116] Wen J, Song J, Bai Y, Liu Y, Cai X, Mei L, *et al*. A Model of Waardenburg Syndrome Using Patient-Derived iPSCs With a *SOX10* Mutation Displays Compromised Maturation and Function of the Neural Crest That Involves Inner Ear Development. Frontiers in Cell and Developmental Biology. 2021; 9: 720858.
[117] Kessler S, Minoux M, Joshi O, Ben Zouari Y, Ducret S, Ross F, *et al*. A multiple super-enhancer region establishes inter-TAD interactions and controls Hoxa function in cranial neural crest. Nature Communications. 2023; 14: 3242.
[118] Sharpe PT. Neural crest and tooth morphogenesis. Advances in Dental Research. 2001; 15: 4–7.
[119] Cobourne MT, Mitsiadis T. Neural crest cells and patterning of the mammalian dentition. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution. 2006; 306: 251–260.
[120] Sharpe PT. Homeobox genes and orofacial development. Connective Tissue Research. 1995; 32: 17–25.
[121] James CT, Ohazama A, Tucker AS, Sharpe PT. Tooth development is independent of a Hox patterning programme. Developmental Dynamics. 2002; 225: 332–335.
[122] Thesleff I, Sharpe P. Signalling networks regulating dental development. Mechanisms of Development. 1997; 67: 111–123.
[123] Tucker AS, Al Khamis A, Sharpe PT. Interactions between Bmp-4 and Msx-1 act to restrict gene expression to odontogenic mesenchyme. Developmental Dynamics. 1998; 212: 533–539.
[124] Plein A, Fantin A, Ruhrberg C. Neural crest cells in cardiovas-

cular development. Current Topics in Developmental Biology. 2015; 111: 183–200.
[125] Miyagawa-Tomita S, Waldo K, Tomita H, Kirby ML. Temporospatial study of the migration and distribution of cardiac neural crest in quail-chick chimeras. The American Journal of Anatomy. 1991; 192: 79–88.
[126] Zhao J, Mommersteeg MTM. Slit-Robo signalling in heart development. Cardiovascular Research. 2018; 114: 794–804.
[127] Diman NYSG, Remacle S, Bertrand N, Picard JJ, Zaffran S, Rezsohazy R. A retinoic acid responsive Hoxa3 transgene expressed in embryonic pharyngeal endoderm, cardiac neural crest and a subdomain of the second heart field. PloS One. 2011; 6: e27624.
[128] Kameda Y. Hoxa3 and signaling molecules involved in aortic arch patterning and remodeling. Cell and Tissue Research. 2009; 336: 165–178.
[129] Brown CB, Feiner L, Lu MM, Li J, Ma X, Webber AL, *et al*. PlexinA2 and semaphorin signaling during cardiac neural crest development. Development. 2001; 128: 3071–3080.
[130] Kameyama T, Murakami Y, Suto F, Kawakami A, Takagi S, Hirata T, *et al*. Identification of plexin family molecules in mice. Biochemical and Biophysical Research Communications. 1996; 226: 396–402.
[131] Stottmann RW, Choi M, Mishina Y, Meyers EN, Klingensmith J. BMP receptor IA is required in mammalian neural crest cells for development of the cardiac outflow tract and ventricular myocardium. Development. 2004; 131: 2205–2218.
[132] Aybar MJ, Mayor R. Early induction of neural crest cells: lessons learned from frog, fish and chick. Current Opinion in Genetics & Development. 2002; 12: 452–458.
[133] Solloway MJ, Harvey RP. Molecular pathways in myocardial development: a stem cell perspective. Cardiovascular Research. 2003; 58: 264–277.
[134] Monzen K, Nagai R, Komuro I. A role for bone morphogenetic protein signaling in cardiomyocyte differentiation. Trends in Cardiovascular Medicine. 2002; 12: 263–269.
[135] Alexander BE, Zhao H, Astrof S. SMAD4: A critical regulator of cardiac neural crest cell fate and vascular smooth muscle development. Developmental Dynamics. 2024; 253: 119–143.
[136] Luo JY, Zhang Y, Wang L, Huang Y. Regulators and effectors of bone morphogenetic protein signalling in the cardiovascular system. The Journal of Physiology. 2015; 593: 2995–3011.
[137] Yanagisawa H, Hammer RE, Richardson JA, Williams SC, Clouthier DE, Yanagisawa M. Role of Endothelin-1/Endothelin-A receptor-mediated signaling pathway in the aortic arch patterning in mice. The Journal of Clinical Investigation. 1998; 102: 22–33.
[138] Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nature Genetics. 1994; 7: 353–361.
[139] Huang GY, Cooper ES, Waldo K, Kirby ML, Gilula NB, Lo CW. Gap junction-mediated cell-cell communication modulates mouse neural crest migration. The Journal of Cell Biology. 1998; 143: 1725–1734.
[140] Seo S, Kume T. Forkhead transcription factors, Foxc1 and Foxc2, are required for the morphogenesis of the cardiac outflow tract. Developmental Biology. 2006; 296: 421–436.
[141] Gammill LS, Roffers-Agarwal J. Division of labor during trunk neural crest development. Developmental Biology. 2010; 344: 555–565.
[142] Müller F, Rohrer H. Molecular control of ciliary neuron development: BMPs and downstream transcriptional control in the parasympathetic lineage. Development. 2002; 129: 5707–5717.
[143] Lee HY, Kléber M, Hari L, Brault V, Suter U, Taketo MM, *et al*. Instructive role of Wnt/beta-catenin in sensory fate specification in neural crest stem cells. Science (New York, N.Y.). 2004; 303: 1020–1023.
[144] Herbarth B, Pingault V, Bondurand N, Kuhlbrodt K, Hermans-Borgmeyer I, Puliti A, *et al*. Mutation of the Sry-related Sox10 gene in Dominant megacolon, a mouse model for human Hirschsprung disease. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95: 5161–5165.
[145] Aoki Y, Saint-Germain N, Gyda M, Magner-Fink E, Lee YH, Credidio C, *et al*. Sox10 regulates the development of neural crest-derived melanocytes in Xenopus. Developmental Biology. 2003; 259: 19–33.
[146] Bahmad HF, Thiravialingam A, Sriganeshan K, Gonzalez J, Alvarez V, Ocejo S, *et al*. Clinical Significance of SOX10 Expression in Human Pathology. Current Issues in Molecular Biology. 2023; 45: 10131–10158.
[147] Le Meur N, Holder-Espinasse M, Jaillard S, Goldenberg A, Joriot S, Amati-Bonneau P, *et al*. MEF2C haploinsufficiency caused by either microdeletion of the 5q14.3 region or mutation is responsible for severe mental retardation with stereotypic movements, epilepsy and/or cerebral malformations. Journal of Medical Genetics. 2010; 47: 22–29.
[148] Saito D, Takase Y, Murai H, Takahashi Y. The dorsal aorta initiates a molecular cascade that instructs sympatho-adrenal specification. Science (New York, N.Y.). 2012; 336: 1578–1581.
[149] Ernsberger U, Patzke H, Tissier-Seta JP, Reh T, Goridis C, Rohrer H. The expression of tyrosine hydroxylase and the transcription factors cPhox-2 and Cash-1: evidence for distinct inductive steps in the differentiation of chick sympathetic precursor cells. Mechanisms of Development. 1995; 52: 125–136.
[150] Varley JE, Maxwell GD. BMP-2 and BMP-4, but not BMP-6, increase the number of adrenergic cells which develop in quail trunk neural crest cultures. Experimental Neurology. 1996; 140: 84–94.
[151] Rohrer H. Transcriptional control of differentiation and neurogenesis in autonomic ganglia. The European Journal of Neuroscience. 2011; 34: 1563–1573.
[152] Tsarovina K, Pattyn A, Stubbusch J, Müller F, van der Wees J, Schneider C, *et al*. Essential role of Gata transcription factors in sympathetic neuron development. Development. 2004; 131: 4775–4786.
[153] Hirsch MR, Tiveron MC, Guillemot F, Brunet JF, Goridis C. Control of noradrenergic differentiation and Phox2a expression by MASH1 in the central and peripheral nervous system. Development. 1998; 125: 599–608.
[154] Furlan A, Lübke M, Adameyko I, Lallemend F, Ernfors P. The transcription factor Hmx1 and growth factor receptor activities control sympathetic neurons diversification. The EMBO Journal. 2013; 32: 1613–1625.
[155] Shtukmaster S, Huber K. The role of the Notch signalling pathway in regulating the balance between neuronal and nonneuronal cells in sympathetic ganglia and the adrenal gland. PloS One. 2023; 18: e0281486.
[156] Schwarz Q, Ruhrberg C. Neuropilin, you gotta let me know: should I stay or should I go? Cell Adhesion & Migration. 2010; 4: 61–66.
[157] Erickson CA, Duong TD, Tosney KW. Descriptive and experimental analysis of the dispersion of neural crest cells along the dorsolateral path and their entry into ectoderm in the chick embryo. Developmental Biology. 1992; 151: 251–272.
[158] Harris ML, Hall R, Erickson CA. Directing pathfinding along the dorsolateral path - the role of EDNRB2 and EphB2 in overcoming inhibition. Development. 2008; 135: 4113–4122.
[159] De Bellard ME, Ortega B, Sao S, Kim L, Herman J, Zuhdi N. Neuregulin-1 is a chemoattractant and chemokinetic molecule for trunk neural crest cells. Developmental Dynamics. 2018; 247: 888–902.
[160] Miller RJ, Banisadr G, Bhattacharyya BJ. CXCR4 signaling in

the regulation of stem cell migration and development. Journal of Neuroimmunology. 2008; 198: 31–38.
[161] Belmadani A, Tran PB, Ren D, Assimacopoulos S, Grove EA, Miller RJ. The chemokine stromal cell-derived factor-1 regulates the migration of sensory neuron progenitors. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2005; 25: 3995–4003.
[162] Stubbusch J, Narasimhan P, Huber K, Unsicker K, Rohrer H, Ernsberger U. Synaptic protein and pan-neuronal gene expression and their regulation by Dicer-dependent mechanisms differ between neurons and neuroendocrine cells. Neural Development. 2013; 8: 16.
[163] Hearn CJ, Murphy M, Newgreen D. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. Developmental Biology. 1998; 197: 93–105.
[164] Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, *et al*. GDNF signalling through the Ret receptor tyrosine kinase. Nature. 1996; 381: 789–793.
[165] Waldo KL, Lo CW, Kirby ML. Connexin 43 expression reflects neural crest patterns during cardiovascular development. Developmental Biology. 1999; 208: 307–323.
[166] Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, *et al*. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell. 1994; 79: 1257–1266.
[167] Murisier F, Guichard S, Beermann F. The tyrosinase enhancer is activated by Sox10 and Mitf in mouse melanocytes. Pigment Cell Research. 2007; 20: 173–184.
[168] Ludwig A, Rehberg S, Wegner M. Melanocyte-specific expression of dopachrome tautomerase is dependent on synergistic gene activation by the Sox10 and Mitf transcription factors. FEBS Letters. 2004; 556: 236–244.
[169] Bondurand N, Pingault V, Goerich DE, Lemort N, Sock E, Le Caignec C, *et al*. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Human Molecular Genetics. 2000; 9: 1907–1917.
[170] Lacosta AM, Muniesa P, Ruberte J, Sarasa M, Domínguez L. Novel expression patterns of Pax3/Pax7 in early trunk neural crest and its melanocyte and non-melanocyte lineages in amniote embryos. Pigment Cell Research. 2005; 18: 243–251.
[171] Curran K, Raible DW, Lister JA. Foxd3 controls melanophore specification in the zebrafish neural crest by regulation of Mitf. Developmental Biology. 2009; 332: 408–417.
[172] Agarwal P, Verzi MP, Nguyen T, Hu J, Ehlers ML, McCulley DJ, *et al*. The MADS box transcription factor MEF2C regulates melanocyte development and is a direct transcriptional target and partner of SOX10. Development. 2011; 138: 2555–2565.
[173] Black BL, Cripps RM. Chapter 9.5 - Myocyte Enhancer Factor 2 Transcription Factors in Heart Development and Disease (pp. 673–99). In Rosenthal N, Harvey RP (eds.) Heart Development and Regeneration. Academic Press: Boston. 2010.
[174] Abu-Elmagd M, Garcia-Morales C, Wheeler GN. Frizzled7 mediates canonical Wnt signaling in neural crest induction. Developmental Biology. 2006; 298: 285–298.
[175] Dorsky RI, Moon RT, Raible DW. Control of neural crest cell fate by the Wnt signalling pathway. Nature. 1998; 396: 370–373.
[176] Saito H, Yasumoto KI, Takeda K, Takahashi K, Fukuzaki A, Orikasa S, *et al*. Melanocyte-specific microphthalmia-associated transcription factor isoform activates its own gene promoter through physical interaction with lymphoid-enhancing factor 1. The Journal of Biological Chemistry. 2002; 277: 28787–28794.
[177] Sato S, Roberts K, Gambino G, Cook A, Kouzarides T, Goding CR. CBP/p300 as a co-factor for the Microphthalmia transcription factor. Oncogene. 1997; 14: 3083–3092.
[178] Keshet E, Lyman SD, Williams DE, Anderson DM, Jenkins NA, Copeland NG, *et al*. Embryonic RNA expression patterns of the c-kit receptor and its cognate ligand suggest multiple functional roles in mouse development. The EMBO Journal. 1991; 10: 2425–2435.
[179] Matsui Y, Zsebo KM, Hogan BL. Embryonic expression of a haematopoietic growth factor encoded by the Sl locus and the ligand for c-kit. Nature. 1990; 347: 667–669.
[180] Nocka K, Majumder S, Chabot B, Ray P, Cervone M, Bernstein A, *et al*. Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice–evidence for an impaired c-kit kinase in mutant mice. Genes & Development. 1989; 3: 816–826.
[181] Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, *et al*. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell. 1994; 79: 1277–1285.
[182] Imokawa G, Kobayasi T, Miyagishi M. Intracellular signaling mechanisms leading to synergistic effects of endothelin-1 and stem cell factor on proliferation of cultured human melanocytes. Cross-talk via trans-activation of the tyrosine kinase c-kit receptor. The Journal of Biological Chemistry. 2000; 275: 33321–33328.
[183] Kuo BR, Erickson CA. Regional differences in neural crest morphogenesis. Cell Adhesion & Migration. 2010; 4: 567–585.
[184] Nagy N, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. Seminars in Cell & Developmental Biology. 2017; 66: 94–106.
[185] Espinosa-Medina I, Jevans B, Boismoreau F, Chettouh Z, Enomoto H, Müller T, *et al*. Dual origin of enteric neurons in vagal Schwann cell precursors and the sympathetic neural crest. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114: 11980–11985.
[186] Cui J, Michaille JJ, Jiang W, Zile MH. Retinoid receptors and vitamin A deficiency: differential patterns of transcription during early avian development and the rapid induction of RARs by retinoic acid. Developmental Biology. 2003; 260: 496–511.
[187] Simkin JE, Zhang D, Rollo BN, Newgreen DF. Retinoic acid upregulates ret and induces chain migration and population expansion in vagal neural crest cells to colonise the embryonic gut. PloS One. 2013; 8: e64077.
[188] Heuckeroth RO, Lampe PA, Johnson EM, Milbrandt J. Neurturin and GDNF promote proliferation and survival of enteric neuron and glial progenitors in vitro. Developmental Biology. 1998; 200: 116–129.
[189] Akbareian SE, Nagy N, Steiger CE, Mably JD, Miller SA, Hotta R, *et al*. Enteric neural crest-derived cells promote their migration by modifying their microenvironment through tenascin-C production. Developmental Biology. 2013; 382: 446–456.
[190] Grumet M, Hoffman S, Crossin KL, Edelman GM. Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. Proceedings of the National Academy of Sciences of the United States of America. 1985; 82: 8075–8079.
[191] Tucker RP. Abnormal neural crest cell migration after the in vivo knockdown of tenascin-C expression with morpholino antisense oligonucleotides. Developmental Dynamics. 2001; 222: 115–119.
[192] Probstmeier R, Pesheva P. Tenascin-C inhibits beta1 integrin-dependent cell adhesion and neurite outgrowth on fibronectin by a disialoganglioside-mediated signaling mechanism. Glycobiology. 1999; 9: 101–114.
[193] Breau MA, Dahmani A, Broders-Bondon F, Thiery JP, Dufour S. Beta1 integrins are required for the invasion of the caecum and proximal hindgut by enteric neural crest cells. Development.

2009; 136: 2791–2801.
[194] McKeown SJ, Chow CW, Young HM. Development of the submucous plexus in the large intestine of the mouse. Cell and Tissue Research. 2001; 303: 301–305.
[195] Huycke TR, Miller BM, Gill HK, Nerurkar NL, Sprinzak D, Mahadevan L, *et al*. Genetic and Mechanical Regulation of Intestinal Smooth Muscle Development. Cell. 2019; 179: 90–105.e21.
[196] Walton KD, Gumucio DL. Hedgehog Signaling in Intestinal Development and Homeostasis. Annual Review of Physiology. 2021; 83: 359–380.
[197] Li Z, Ngan ESW. New insights empowered by single-cell sequencing: From neural crest to enteric nervous system. Computational and Structural Biotechnology Journal. 2022; 20: 2464–2472.
[198] Lasrado R, Boesmans W, Kleinjung J, Pin C, Bell D, Bhaw L, *et al*. Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. Science (New York, N.Y.). 2017; 356: 722–726.
[199] Hao MM, Young HM. Development of enteric neuron diversity. Journal of Cellular and Molecular Medicine. 2009; 13: 1193–1210.
[200] Medina-Cuadra L, Monsoro-Burq AH. Xenopus, an emerging model for studying pathologies of the neural crest. Current Topics in Developmental Biology. 2021; 145: 313–348.
[201] Le Douarin NM. The avian embryo as a model to study the development of the neural crest: a long and still ongoing story. Mechanisms of Development. 2004; 121: 1089–1102.
[202] Barriga EH, Trainor PA, Bronner M, Mayor R. Animal models for studying neural crest development: is the mouse different? Development. 2015; 142: 1555–1560.
[203] Katouni K, Nikolaou A, Mariolis T, Protogerou V, Chrysikos D, Theofilopoulou S, *et al*. Syndromic Craniosynostosis: A Comprehensive Review. Cureus. 2023; 15: e50448.
[204] Chen ZF, Behringer RR. twist is required in head mesenchyme for cranial neural tube morphogenesis. Genes & Development. 1995; 9: 686–699.
[205] Santagati F, Minoux M, Ren SY, Rijli FM. Temporal requirement of Hoxa2 in cranial neural crest skeletal morphogenesis. Development. 2005; 132: 4927–4936.
[206] Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, *et al*. Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron. 1997; 19: 27–37.
[207] Beverdam A, Merlo GR, Paleari L, Mantero S, Genova F, Barbieri O, *et al*. Jaw transformation with gain of symmetry after Dlx5/Dlx6 inactivation: mirror of the past? Genesis (New York, N.Y.: 2000). 2002; 34: 221–227.
[208] Depew MJ, Lufkin T, Rubenstein JLR. Specification of jaw subdivisions by Dlx genes. Science (New York, N.Y.). 2002; 298: 381–385.
[209] Landsend ECS, Lagali N, Utheim TP. Congenital aniridia - A comprehensive review of clinical features and therapeutic approaches. Survey of Ophthalmology. 2021; 66: 1031–1050.
[210] Keyte A, Hutson MR. The neural crest in cardiac congenital anomalies. Differentiation; Research in Biological Diversity. 2012; 84: 25–40.
[211] Théveniau-Ruissy M, Dandonneau M, Mesbah K, Ghez O, Mattei MG, Miquerol L, *et al*. The del22q11.2 candidate gene Tbx1 controls regional outflow tract identity and coronary artery patterning. Circulation Research. 2008; 103: 142–148.
[212] Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, *et al*. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Developmental Cell. 2003; 5: 45–57.
[213] Jia Q, McDill BW, Li SZ, Deng C, Chang CP, Chen F. Smad signaling in the neural crest regulates cardiac outflow tract remodeling through cell autonomous and non-cell autonomous effects. Developmental Biology. 2007; 311: 172–184.
[214] Nie X, Deng CX, Wang Q, Jiao K. Disruption of Smad4 in neural crest cells leads to mid-gestation death with pharyngeal arch, craniofacial and cardiac defects. Developmental Biology. 2008; 316: 417–430.
[215] Wang J, Xu N, Feng X, Hou N, Zhang J, Cheng X, *et al*. Targeted disruption of Smad4 in cardiomyocytes results in cardiac hypertrophy and heart failure. Circulation Research. 2005; 97: 821–828.
[216] Dudas M, Sridurongrit S, Nagy A, Okazaki K, Kaartinen V. Craniofacial defects in mice lacking BMP type I receptor Alk2 in neural crest cells. Mechanisms of Development. 2004; 121: 173–182.
[217] Zhao J, Zhu Y, Xie X, Yao Y, Zhang J, Zhang R, *et al*. Pleiotropic effect of common *PHOX2B* variants in Hirschsprung disease and neuroblastoma. Aging. 2019; 11: 1252–1261.
[218] Dermitzakis I, Manthou ME, Meditskou S, Miliaras D, Kesidou E, Boziki M, *et al*. Developmental Cues and Molecular Drivers in Myelinogenesis: Revisiting Early Life to Re-Evaluate the Integrity of CNS Myelin. Current Issues in Molecular Biology. 2022; 44: 3208–3237.
[219] Theotokis P. Exploring Myelin Dynamics in Demyelinating Disorders at the Molecular Level. Current Issues in Molecular Biology. 2024; 46: 1754–1756.
[220] Zhu Q, Lu Q, Gao R, Cao T. Prospect of Human Pluripotent Stem Cell-Derived Neural Crest Stem Cells in Clinical Application. Stem Cells International. 2016; 2016: 7695836.
[221] Chatzi D, Kyriakoudi SA, Dermitzakis I, Manthou ME, Meditskou S, Theotokis P. Clinical and Genetic Correlation in Neurocristopathies: Bridging a Precision Medicine Gap. Journal of Clinical Medicine. 2024; 13: 2223.
[222] Srinivasan A, Toh YC. Human Pluripotent Stem Cell-Derived Neural Crest Cells for Tissue Regeneration and Disease Modeling. Frontiers in Molecular Neuroscience. 2019; 12: 39.
[223] Kumar KR, Cowley MJ, Davis RL. Next-Generation Sequencing and Emerging Technologies. Seminars in Thrombosis and Hemostasis. 2019; 45: 661–673.